Modelling the Fate of Xenobiotic Trace Chemicals via Wastewater Treatment and Agricultural Resource Reuse

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DTU Environment
Department of Environmental Engineering

PhD Thesis
January 2016

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Preface

This thesis summarizes the results of a PhD project carried out at the Technical University of Denmark, Department of Environmental Engineering, from April 2012 to January 2016. The project was supervised by Assoc. Prof. Benedek Gy. Plósz, Prof. Stefan Trapp, and Assoc. Prof. Henrik R. Andersen. Project funding was provided by the Technical University of Denmark, Department of Environmental Engineering.

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the text by their paper number written with the Roman numerals **I-IV**.

I **Polesel, F.**, Lehnberg, K., Dott, W., Trapp, S., Thomas, K.V., Plósz, B.G., 2015. Factors influencing sorption of ciprofloxacin onto activated sludge: Experimental assessment and modelling implications. *Chemosphere* **119**, 105–111.

II **Polesel, F.**, Torresi, E., Loreggian, L., Escolá-Casas, M., Christensson, M., Bester, K., Plósz, B.G. (2015). Elimination of pharmaceuticals in predenitrifying MBBR – Influence of exposure to primary substrate in single-stage and three-stage configurations. *Manuscript in preparation.*

III **Polesel, F.,** Andersen, H.R., Trapp, S., Plósz, B.G. (2015). Removal of antibiotics in biological wastewater treatment systems – A critical assessment of factors using the Activated Sludge Modelling Framework for Xenobiotics (ASM-X). *Submitted manuscript.*

IV **Polesel, F.**, Plósz, B.G., Trapp, S. (2015). From consumption to harvest: Environmental fate prediction of excreted ionizable trace organic chemicals. *Water Research* **84**, 85–98.

In this online version of the thesis, paper **I-IV** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from DTU Environment, Technical University of Denmark, Miljoevej, Building 113, 2800 Kgs. Lyngby, Denmark, info@env.dtu.dk.

In addition, the following publications, not included in this thesis, were also concluded during this PhD study

- Ramin, P., Libonati, A., **Polesel, F.**, Causanilles Llanes, A., Emke, E., de Voogt, P., Plósz, B.G. Sorption and transformation of illicit drug biomarkers in sewer systems: the role of suspended solids in raw wastewater. Manuscript in preparation.

This PhD study also contributed to international conferences with the following proceeding and conference papers:

- **Polesel, F.**, Plósz, B.G., Trapp, S. (2013). Activity-based fate modelling for risk assessment of three ionizable organic compounds (triclosan, furosemide, ciprofloxacin). Micropol & Ecohazard 2013, 8th IWA Specialist Conference on Assessment and Control of Micropollutants and Hazardous Substances in Water, Zurich, Switzerland, 16–23 June 2013.

- **Polesel, F.**, Lehnberg, K., Dott, W., Trapp, S., Thomas, K.V., Plósz, B.G. (2013). Modelling the fate of ciprofloxacin in activated sludge systems - The relevance of the sorption process. ICCE 2013, 14th EuCheMS International Conference on Chemistry and the Environment, Barcelona, Spain, 25–28 June 2013.

- **Polesel, F.**, Lehnberg, K., Dott, W., Trapp, S., Thomas, K.V., Plósz, B.G. (2013). Modelling sorption of ciprofloxacin using the ASM-X framework – Evaluation of factors influencing activated sludge treatment and implications on environmental risk assessment. NORDIWA 2013, 13th Nordic Wastewater Conference, Malmö, Sweden, 8–10 October 2013.

- **Polesel, F.**, Langford, K.H., Trapp, S., Thomas, K.V., Plósz, B.G. (2014). Removal of pharmaceuticals in biological wastewater treatment systems: model generalisation and implications for environmental risk predictions. WWTmod 2014, 4th IWA/WEF Wastewater Treatment Modelling Seminar, Spa, Belgium, 30 March–2 April 2014.

- Ramin, P., **Polesel, F.**, Andresson, G., Vezzaro, K., Sharma, A.K., Reid, M.J., Thomas, K.V., Mikkelsen, P.S., Plósz, B.G. (2014). Impacts of hydraulic residence time prediction and diurnal loading pattern on the estimation of drug abuse in urban areas. ICUD 2014, 13th International Conference on Urban Drainage, Kuching, Malaysia, 7–11 September 2014.

- **Polesel, F.**, Langford, K.H., Trapp, S., Thomas, K.V., Plósz, B.G. (2014). Removal of pharmaceuticals in sewage treatment plants: A model generalisation to international data. 2014 IWA World Water Congress & Exhibition, Lisbon, Portugal, 21–26 September 2014.
- Ramin, P., Causanilles, A., **Polesel, F.**, Emke, E., de Voogt, P., Plósz, B.G. (2015). Abiotic and biofilm-mediated transformation of heroin biomarkers in wastewater under aerobic and anaerobic conditions. Testing the Waters, 2nd International Conference on Wastewater-based drug epidemiology, Ascona, Switzerland, 11–15 October 2015.
- Plósz, B.G., **Polesel, F.** (2015). Characterising the removal of trace organic chemicals in wastewater – Are we using the right tools? Micropol & Ecohazard 2015, 9th IWA Specialist Conference on Assessment and Control of Micropollutants and Hazardous Substances in Water, Singapore, Singapore, 22–25 November 2015.
- **Polesel, F.**, Torresi, E., Loreggian, Escolá Casa, M., Bester, K., Plósz, B.G. (2015). Elimination of pharmaceuticals in single- and three-stage predenitrifying MBBR. Micropol & Ecohazard 2015, 9th IWA Specialist Conference on Assessment and Control of Micropollutants and Hazardous Substances in Water, Singapore, Singapore, 22–25 November 2015.
- **Polesel, F.**, Plósz, B.G., Trapp, S. (2015). Modelling the fate of ionizable trace organic chemicals from consumption to food crops. Micropol & Ecohazard 2015, 9th IWA Specialist Conference on Assessment and Control of Micropollutants and Hazardous Substances in Water, Singapore, Singapore, 22–25 November 2015.

Acknowledgements

It is 6.19 AM and in a few hours I will (hopefully) hand in this PhD thesis. At this time of a (long) Danish night it is challenging for me to remember everyone standing by my side during this 3-year-and-something-long adventure as PhD student. Therefore, I apologize if I will forget to thank any of you—please understand me.

First and foremost, I would like to thank Assoc. Prof. Benedek Plósz for giving me the opportunity of carrying out my PhD studies under his supervision and continuing his previous research, for his contagious enthusiasm, for his continuous brainstorming of ideas and, overall, for his constant support to my research activities (even at the oddest hours of the day). I am also very grateful to Prof. Stefan Trapp for being more than a co-supervisor, for welcoming me as full member of his research group, for the critical discussions about research and for the informal lectures about trees, but essentially for being there during the few breakdowns of these last years. Eventually, I would like to thank Assoc. Prof. Henrik Andersen for his precious pieces of advice during my start-up with laboratory activities.

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Summary

As a result of widespread human activities, pharmaceuticals and biocides are ubiquitously present at trace levels in the environment. Large amounts of these substances, also identified as xenobiotic trace chemicals (XTCs), are released daily from: (i) households and healthcare facilities, following human consumption and disposal; (ii) husbandry and other analogous facilities, following veterinary consumption; and (iii) industrial facilities. A significant fraction of these emissions reaches municipal wastewater treatment plants (WWTPs), where XTCs undergo incomplete removal partly due to WWTP design limitations. These chemicals are thus eventually released to the environment, e.g. in freshwater bodies receiving WWTP effluents, representing a threat to living organisms.

WWTPs have been generally identified as a major point source of XTC emissions to the environment. Nevertheless, due to the high number of marketed and consumed chemicals, and to the uncertainties associated to sampling and analytical methodologies, quantifying the elimination of XTCs during wastewater treatment still remains a challenge. Developing robust modelling tools to predict the fate of XTCs in WWTPs can help overcoming this challenge. However, in-depth understanding of mechanisms and processes, determining XTCs removal during wastewater treatment, is still required.

This PhD thesis aimed at filling knowledge gaps in the field of XTC fate modelling during and beyond wastewater treatment. We aimed at improving the comprehension of XTC fate, and thus the predictive capabilities of fate models: (i) at process scale, with a focus on sorption and biological transformation of XTCs in biological treatment systems; (ii) in full-scale WWTPs, assessing the impact of retransformation and WWTP operation on XTC elimination; and (iii) in integrated WWTP-agricultural systems. Different modelling tools, suiting the specific purposes of our investigations, were developed, extended and/or innovatively applied. Fate models used as reference in this thesis include: the Activated Sludge Modelling framework for Xenobiotics (ASM-X); the generic WWTP model SimpleTreat Activity; and the dynamic soil-plant model for fate prediction in agricultural systems.

Experimental and model-based observations were combined to assess sorption of ionizable XTCs onto activated sludge and XTC biotransformation in moving bed biofilm reactors (MBBRs). Most XTCs are in fact multispecies

chemicals, being present in neutral and/or ionized form in wastewater. We demonstrated that pH conditions and, to a lesser extent, iron salt dosing for chemical phosphorus removal can significantly affect solid-liquid partitioning of the zwitterionic antibiotic ciprofloxacin onto activated sludge. Electrostatic interactions and complexation are thus dominating sorption mechanisms. Under a range of pH, redox and iron salt dosing conditions, non-linear sorption (n=0.62–1.33) was observed. Extensions to traditional partitioning models were accordingly proposed for ciprofloxacin and other zwitterionic XTCs, accounting for: (i) high non-linearity of XTC sorption; or (ii) ionization with changing pH and different sorption potential of ionized species. Furthermore, XTCs are typically present in ng L^{-1} to µg L^{-1} concentrations in wastewater, being referred to as non-growth substrates, and their biological degradation can be associated with microbial growth processes. In this PhD thesis, we assessed the influence of primary metabolic processes on XTC biotransformation in MBBR biofilm. Our investigation was performed by comparing biotransformation kinetics in pre-denitrifying MBBRs operated in single-stage and three-stage configurations. The latter configuration produced a prolonged biofilm exposure to organic electron donor (COD) loading and complexity tiered by segregated and integrated biofilm reactors, which significantly influenced kinetics of heterotrophic denitrification and XTC biotransformation. Biotransformation rate constants for a number of non-recalcitrant XTCs were found correlated to the denitrification potential of MBBR biofilm, suggesting that XTC degradation occurred via microbial co-metabolism. In addition, enhanced biotransformation kinetics was shown for a number of XTCs (sulfamethoxazole, erythromycin, atenolol) as compared to previous findings for conventional activated sludge.

A number of factors have been described to influence the elimination of XTCs in full-scale WWTPs. Specifically, relevant impact was attributed to (i) solid residence time (SRT), at which biological treatment is operated; and (ii) the formation of XTCs due to, e.g., deconjugation of human metabolites. Many XTCs are in fact excreted by humans in the form of conjugates, which can undergo biotic retransformation to parent chemicals. In this PhD thesis, we specifically assessed the influence of retransformation processes and SRT on the fate of sulfamethoxazole in full-scale WWTPs. A methodology based on the comparison of ASM-X predictions and literature data was used. We demonstrated that the impact of retransformation during secondary wastewater treatment is determined by: (i) the size of WWTP catchments,

with major in-sewer retransformation expected in large catchments; (ii) the type of catchment (hospital or urban catchment). This evidence accordingly suggests an integrated approach to XTC fate assessment in wastewater systems (sewer networks and WWTPs). Furthermore, improved elimination of sulfamethoxazole was found and predicted in WWTPs operated at SRT greater than 16 d. Beyond this critical SRT, enhanced biotransformation kinetics may occur due to the enrichment of slow-growing organisms (e.g., specialist degraders) or mixed substrate utilization strategies. This finding supported our experimental evidence of enhanced sulfamethoxazole biotransformation kinetics in denitrifying MBBRs.

As a result of incomplete biodegradation in WWTPs, XTCs persist in effluents and sewage sludge. Reuse of municipal biosolids and treated wastewater or use of freshwater for agricultural purposes eventually leads to XTC uptake into food crops. In this PhD thesis, we developed and tested a generic simulation tool to predict the fate of XTCs from consumption, through wastewater treatment and eventually to the uptake by winter wheat for a number of geographical scenarios in the European Union. The tool combined was specifically addressed for fate prediction of ionizable XTCs (the biocide triclosan, the diuretic furosemide and the antibiotic ciprofloxacin). Furosemide was found rather persistent to wastewater treatment (removal efficiency $\leq$ 40%) and to further undergo significant accumulation in wheat. Uptake of furosemide was predicted to increase (+20% of emissions to soil) when emissions to the soil-plant system occurred via freshwater irrigation, as compared to soil amendment with biosolids. Due to the scarce availability of experimental data, our model predictions indicate the need of deepening investigations of XTC fate in agricultural systems. Accumulation in food crops may result in indirect human exposure to XTCs via dietary intake, which can be eventually estimated using model predictions. The presented simulation tool can thus be used for pre-screening and priority setting of chemicals, and to explore the impact of additional XTC emission pathways (e.g., manure application, irrigation with reclaimed WWTP effluent) in terms of food crop accumulation.

Dansk sammenfatning

Som et resultat af menneskelige aktiviteter er lægemidler og biocider allestedsnærværende i miljøet på sporstofniveau. Store mængder af disse stoffer, også kendt som miljøfremmede stoffer (på Engelsk XTCs: Xenobiotic Trace Chemicals), frigives dagligt fra: (i) husholdninger og sundhedsfaciliteter, som følge af indtagelse og bortskaffelse; (ii) dyrehold og tilsvarende faciliteter, som følge af dyrs indtagelse; og (iii) industrianlæg. En betydelig del af disse udledninger når frem til kommunale renseanlæg, hvor de miljøfremmede stoffer kun delvist bliver fjernet. I sidste ende udledes de miljøfremmede stoffer således til miljøet, f.eks. til ferskvandsområder der fungerer som recipienter for renseanlæg, hvor de udgør en trussel for levende organismer.

Renseanlæg er blevet identificeret som en vigtig punktkilde for udledning af miljøfremmede stoffer til miljøet. På grund af det høje antal af markedsførte og forbrugt kemikalier, og de usikkerheder forbundet til prøveudtagning og analysemetoder, er der dog stadig store udfordringer forbundet med kvantificering af i hvor høj grad miljøfremmede stoffer bliver fjernet i forbindelse spildevandsrensning. Udvikling af robuste modelleringsværktøjer til at forudsige miljøfremmede stoffers skæbne i renseanlæg kan hjælpe med at overvinde denne udfordring. Men dybtgående forståelse af de mekanismer og processer, der styrer miljøfremmede stoffers fjernelse under spildevandsrensning, er stadig nødvendig.

Målet med nærværende ph.d.-projekt var at udfylde huller i vores viden omkring modellering af miljøfremmed stoffers skæbne i renseanlæg og efterfølgende. Vi ville gerne forbedre forståelsen af miljøfremmede stoffers skæbne og dermed forbedre modellernes forudsigelsesevne: (i) ved processkalaen, med fokus på sorption og biologisk nedbrydning af miljøfremmede stoffer i biologiske renseanlæg; (ii) i fuldskala renseanlæg, hvor vi ville vurdere effekten af gendannelse og driften af renseanlægget på fjernelsesgraden; og (iii) i integrerede renseanlæg-landbrugssystemer. Forskellige modelleringsværktøjer, velegnede til hvert specifikt formål i undersøgelser, blev udviklet, udvidet eller anvendt innovativt. Følgende velkendte modeller blev anvendt: Activated Sludge Modelling framework for Xenobiotics (ASM-X); den generiske renseanlægsmodel SimpleTreat Activity; og den dynamiske jord-plante model til forudsigelse af kemikaliers skæbne i landbrugssystemer.

Vi kombinerede eksperimentelle og modelbaserede observationer til at vurdere sorption af ioniserbare miljøfremmede stoffer til aktiveret slam og nedbrydning af miljøfremmede stoffer i ”moving bed”-biofilmreaktorer (MBBRs). De fleste

miljøfremmede stoffer er ioniserbare, og kan således være til stede i neutral og / eller ioniseret form i spildevand. Vi viste, at pH-betingelser, og i mindre grad, dosering af jernsalt til kemisk phosphorfjernelse, med sikkerhed påvirker faststof-væskepartitioneringen af det zwitterioniske antibiotikum ciprofloxacin i aktivt slam. Elektrostatiske interaktioner og kompleksdannelse er således dominerende sorptionsmekanismer. Vi observerede non-lineær sorption (n=0.62–1.33) under forskellige pH-, redox- og jernsalt doseringsforhold. Vi foreslog derfor en udvidelse til traditionelle sorptionsmodeller for ciprofloxacin og andre zwitterioniske miljøfremmede stoffer, som beskriver ionisering som funktion af pH og ændret sorptionspotentiale for ioniserede stoffer. Miljøfremmede stoffer findes typisk i spildevand i koncetrationsniveauer mellem ng L^{-1} og ug L^{-1}, hvilket medfører at de ikke kan bruges som substrater for vækst. Vi vurderede effekten af primære metaboliske processer i MBBRs på fjernelse af miljøfremmede stoffer. Undersøgelsen blev udført ved at sammenligne kinetikken i pre-denitrificerende MBBRs kørt i enkelttrins- og tretrinskonfigurationer. Sidstnævnte konfiguration produceret en langvarig udsættelse af biofilmen for faldende belastning med COD, hvilket påvirkede kinetikken af heterotrof denitrifikation og nedbrydning af miljøfremmede stoffer. Vi fandt at nedbrydningskonstanten for en række miljøfremmede stoffer var afhængig af denitrifikationspotentialet i biofilmen, hvilket indikerer, at nedbrydning af miljøfremmede stoffer var en co-metabolisk proces.

Der er beskrevet en række faktorer der påvirker fjernelsen af miljøfremmede stoffer i fuldskala renseanlæg. Relevant effekt kan tilskrives (i) faststoffers opholdstid i den biologiske rensningsproces; og (ii) dannelse af miljøfremmede stoffer via f.eks. delvis nedbrydning af menneskelige metabolitter. Mange miljøfremmede stoffer udskilles af mennesker i form af metabolitter, som efterfølgende kan omdannes til moderstoffet igen. I dette projekt vurderede vi effekten af gendannelse og faststofsopholdstid på sulfamethoxazols skæbne i fuldskala renseanlæg. Vi brugte en metode baseret på sammenligning af ASM-X modelforudsigelser med værdier fra litteraturen. Vores undersøgelse viste, at effekten af gendannelse under sekundær spildevandsrensning er bestemt af: (i) størrelsen af renseanlæggets opland, idet høj grad af gendannelse i kloaksystemet kan forventes i store oplande; og (ii) typen af opland (med eller uden hospital). Dette peger på anvendelsen af en integreret tilgang til miljøfremmede stoffers skæbne-vurdering i spildevandsssystemer (kloaksystemer og rensningsanlæg). Endvidere forudså vi og fandt forbedret fjernelse af sulfamethoxazol i renseanlæg der kører med faststofsopholdstider på mere end 16 dage. Ved faststofsopholdstider over denne værdi kan der forventes forbedrede

nedbrydningskinetikker på grund af vækst i langsomtvoksende organismer (f.eks. specialiserede nedbrydere) eller på grund af blandede substratudnyttelsesstrategier.

Miljøfremmede stoffer bliver som regel kun delvist nedbrudt i renseanlæg, og er derfor tilstede i udledninger fra renseanlæg og i spildevandsslammet. Udbringning af slam på marker og brug af renset spildevand eller ferskvand der modtager renset spildevand til kunstvanding af marker medfører i sidste ende optagelse af miljøfremmede stoffer i fødevareafgrøder. I dette projekt udviklede og afprøvede vi et generisk simuleringsværktøj til at forudsige miljøfremmede stoffers skæbne fra forbrug til optagelse i vinterhvede via nedbrydning i renseanlæg i en række geografiske scenarier i EU. Værktøjet kombinerede SimpleTreat Activity-modellen og den dynamiske jord-plante model, med særligt fokus på at forudsige skæbnen af ioniserbare stoffer. Ionisering har, som vist i vores første studie, store konsekvenser på faststof-væske partitioneringen, og på fordelingen ind i kornets væv. Vi udvalgte tre miljøfremmede stoffer som anvendes i store mængder, nemlig biocidet triclosan, det vanddrivende middel furosemid og antibiotikummet ciprofloxacin. Vi valgte tre reelle geografiske scenarier for områder i Den Europæiske Union og brugte gennemsnitlige forbrugsdata eller udledningsværdier fra forskellige EU-lande. Vi fandt at furosemid blev meget svagt nedbrudt i spildevandsrensning og blev signifikant akkumuleret i hvede. Optagelse af furosemid var større når udledning til jord-plante-systemet opstod via kunstvanding med ferskvand, i forhold til udbringning af slam. Set i lyset af de få eksperimentelle data indikerer vores modelberegninger et behov for uddybende undersøgelser af miljøfremmede stoffers skæbne i landbrugssystemer. Ophobning i fødevareafgrøder kan resultere i indirekte eksponering af mennesker for miljøfremmede stoffer via kosten; denne eksponering kan estimeres ved hjælp modelsimuleringer. Det præsenterede simuleringsværktøj kan således anvendes til pre-screening og prioritering af kemikalier samt til at undersøge betydningen af miljøfremmede stoffers emmisionsveje (f.eks. udbringning af gødning irrigation med behandlet spildevand) i forbindelse med akkumulering i fødevareafgrøder.

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Reader's guide

Chapter 1 presents a brief introduction on the main sources and pathways of XTC emission to the environment, describes the motivation and the objectives of this PhD thesis.

Chapters 2 and 3 present an overview of the current knowledge status on the fate of XTCs during wastewater treatment, following the release to sewer systems. A description is provided for: (i) wastewater treatment technologies and configurations, in which XTC removal has been traditionally assessed; (ii) relevant XTC fate processes; and (iii) approaches used and assumptions introduced to model XTC fate (i.e. multimedia activity/fugacity models and concentration-based models).

Chapter 4 focuses on the influence of pH and iron salt dosing on the partitioning of ionizable XTCs onto activated sludge. Results are presented for the antibiotic ciprofloxacin, for which extensions to traditional partitioning models are proposed.

Chapter 5 presents an assessment of XTC biotransformation kinetics in denitrifying moving bed biofilm reactors (MBBRs) in different configurations. Model structures used to describe XTC transformation kinetics are presented. The influence of primary metabolic processes (i.e., heterotrophic denitrification) on XTC biotransformation is eventually discussed.

Chapter 6 discusses the impact of retransformation processes and SRT on the elimination of the antibiotic sulfamethoxazole in full-scale wastewater treatment plants (WWTPs). The discussion is supported by a comparison between removal efficiency measurements from literature and model predictions, using the Activated Sludge Modelling framework for Xenobiotics (ASM-X).

Chapter 7 presents a model-based evaluation of XTC fate from human consumption and excretion, through WWTPs, via irrigation or sewage sludge amendment up to the uptake in food crops at regional level. Plant uptake of three XTCs (triclosan, furosemide and ciprofloxacin) is compared, and the significance of XTC emission pathway to agricultural systems (fertilization or irrigation) is assessed.

Mathematical notations (e.g., XTC concentrations) used in fate models are presented in Chapter 3. Equations in Chapters 2 and 4–6 are written using notation conventions presented for ASM-X.

List of acronyms

A2O	Anaerobic anoxic oxic
ACD	Advanced Chemistry Development
ASM	Activated sludge model
ASM-X	Activated sludge model for Xenobiotics
BCF	Bioconcentration factor
BNR	Biological nutrient removal
BSM1	Benchmark simulation model no. 1
BOD	Biochemical oxygen demand
CAS	Conventional activated sludge
COD	Chemical oxygen demand
DCM	Dissolved and colloidal matter
DNP	Denitrification potential
DO	Dissolved oxygen
E1	Estrone
E2	17-β-estradiol
EBPR	Enhanced biological phosphorus removal
EC	European Community
EEC	European Economic Community
EMEA	European Medicines Evaluation Agency
EPS	Extracellular polymeric substance
EU	European Union
EUSES	European Union System for Evaluation of Substances
FBR	Fixed bed reactor
MBBR	Moving bed biofilm reactor
MBR	Membrane bioreactor
MW	Molecular weight
NADPH	Nicotinamide adenine dinucleotide phosphate

PAH	Polycyclic aromatic hydrocarbons
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SRT	Solid residence time
TSS	Total suspended solids
VSS	Volatile suspended solids
WHO	World Health Organization
WWTP	Wastewater treatment plant
XTC	Xenobiotic trace chemical

List of symbols

0	Referring to uncharged species
+	Referring to cationic species
-	Referring to anionic species
±	Referring to zwitterionic species
a_t	Activity
B	Bulk activity capacity
C	Xenobiotic concentration
C_{LI}	Aqueous parent xenobiotic concentration
C_{CJ}	Aqueous retransformable xenobiotic concentration
C_{SL}	Sorbed parent xenobiotic concentration
D	Octanol-water distribution coefficient
F	Fugacity
F/M	Food-to-microorganism ratio
Fe(III)	Ferric ion
f_{oc}	Fraction of organic carbon
K_S	Half-saturation coefficient (substrate)
K_C	Half-saturation coefficient (xenobiotic)
K_{aw}	Dimensionless air-water partition coefficient
k_{bio}	Biotransformation rate constant
k'_{bio}	Biotransformation rate
k_{dec}	Retransformation rate constant
k_{des}	Desorption rate
K_d	Solid-liquid partition coefficient
K_f	Freundlich partition coefficient
K_I	Inhibition coefficient
K_{oc}	Solid-liquid partition coefficient normalized to organic carbon
K_{ow}	Octanol-water partition coefficient

$K_{a,HSA}$	Partition coefficient to human serum albumin
K_Z	Constant of tautomeric equilibrium
L	Lipid content in plant compartments
M	Mass load
n	Freundlich linearity parameter
$n_{LI,CJ}$	Parent-to-retransformable xenobiotic ratio
NO_3-N	Nitrate concentration as nitrogen
NO_2-N	Nitrite concentration as nitrogen
NO_X	Nitrogen oxide (nitrate and nitrite) concentration as nitrogen
P	Protein content in plant compartments
pK_a	Ionization constant
q	Xenobiotic cometabolic utilization rate constant in the presence of growth substrate
q_C	Xenobiotic cometabolic biotransformation rate constant in the presence of growth substrate
S	Substrate concentration
T	Xenobiotic cometabolic transformation yield
T_{ref}	Reference temperature
X	Concentration of biomass or solids
Y	Microbial growth yield
Z	Fugacity capacity
ϕ	Ionic or neutral fraction
μ_{max}	Maximum microbial growth rate
θ	Arrhenius coefficient
η	Xenobiotic removal efficiency
η_{LI}	Parent-based xenobiotic removal efficiency
η_{TOT}	Total (parent-based and retransformable) xenobiotic removal efficiency

1 Introduction

1.1 Background and problem definition

The general classification of xenobiotic trace chemicals (XTCs) is used to identify man-made chemicals found in the environment, mostly including organic substances such as pharmaceuticals, biocides, illicit drugs, cosmetics, pesticides, herbicides and surfactants. These chemicals are largely products of industrial synthesis processes, and their ubiquitous presence in the environment is essentially the consequence of human activities.

Pharmaceuticals are widely consumed XTCs, finding application in human therapy and animal husbandry (Halling-Sørensen et al., 1998; Boxall et al., 2003; Ternes et al., 2006). Biocides for human use include antimicrobial agents present in a wide range of consumer products (e.g., cosmetics, detergents, clothing), medical products (e.g., disinfectants) and building materials (Singer et al., 2002; Bester, 2003; Bollmann et al., 2014). Release from several sources contributes to their occurrence in the environment, as summarized in Figure 1.1. Following administration to humans and animals, significant fractions of these chemicals are excreted unchanged or in the form of metabolites. Excreted amounts eventually reach municipal wastewater treatment plants (WWTPs) or are directly released to the environment (e.g., via manure application on soil). Additional emissions occur from industrial production sites and from the (also improper) disposal of unutilized XTCs.

In the last three decades, scientific research has focused on the release of pharmaceuticals following human consumption in households and healthcare facilities and on their fate during wastewater treatment. Municipal WWTPs have been accordingly recognized as a major point source of emissions, resulting from limited elimination of trace chemicals (Richardson and Bowron, 1985; Halling-Sørensen et al., 1998; Ternes, 1998; Kümmerer and Henninger, 2003; Ternes et al., 2006). Particular attention has been dedicated to WWTP emissions to freshwater systems (Kolpin et al., 2002; Calamari et al., 2003; Johnson et al., 2008; Kasprzyk-Hordern et al., 2009; Ort et al., 2009).

The environmental presence of pharmaceuticals and biocides at trace concentrations, with consequent living organism exposure, was associated to a number of concerns. Possible effects include: bioaccumulation; acute and chronic toxicity—also as a result of mixture or synergistic effects; and spread of antibiotic resistance (Halling-Sørensen et al., 1998; Jobling et al., 1998; Daughton and Ternes, 1999; Kümmerer and Henninger, 2003; Ternes et al., 2006;

Ågerstrand et al., 2015). Recently, antibiotic resistance was acknowledged as a major future threat for human health (WHO, 2014)

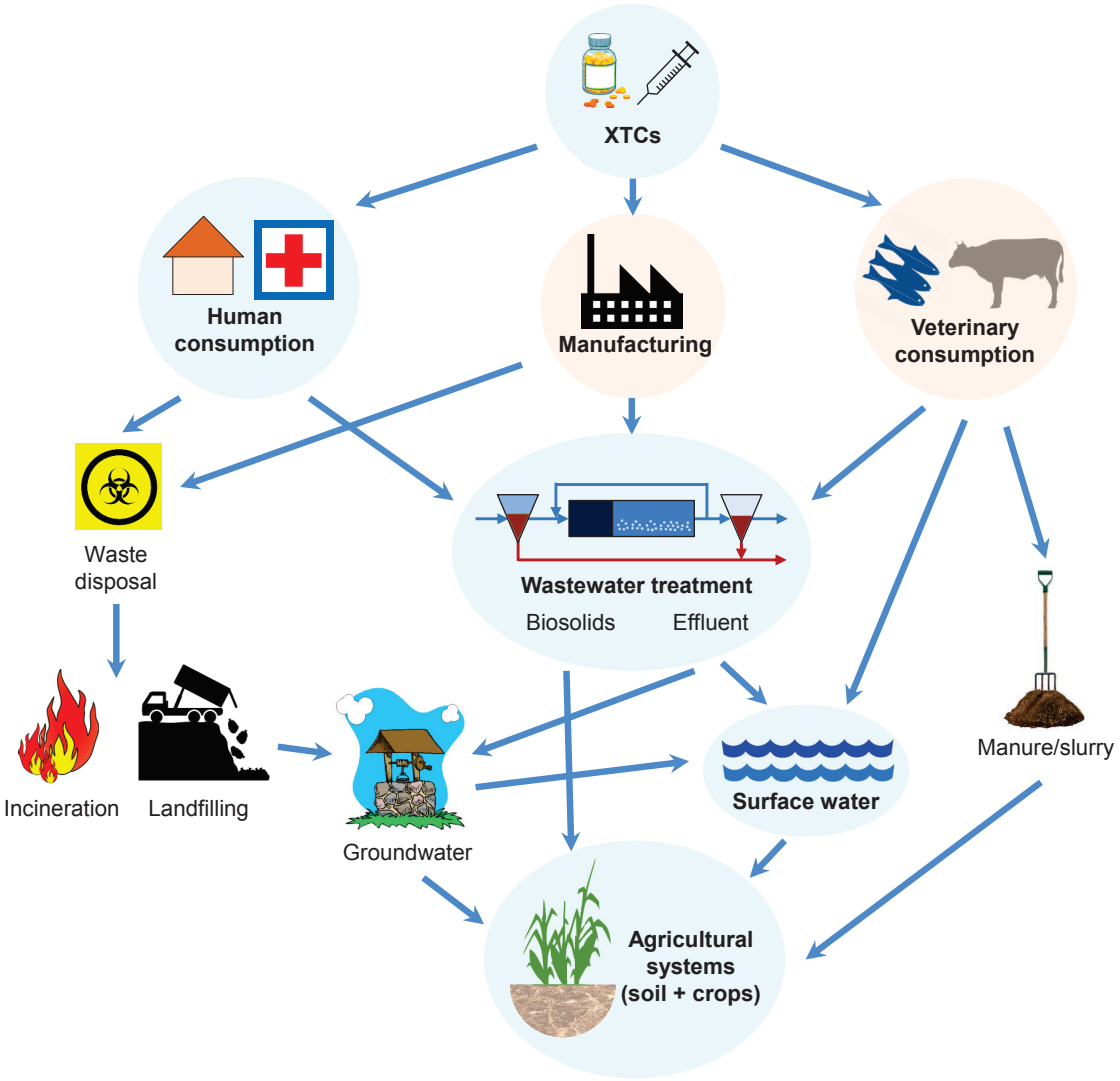


Figure 1.1. Summary of the major sources of consumption and pathways of environmental release for pharmaceuticals and biocides. The research summarized in this PhD thesis primarily focused on sources and recipients highlighted in blue. Although human consumption and excretion was considered as the main emission source, we do not exclude that chemicals assessed in this study were released to WWTPs also by industrial and veterinary facilities (highlighted in pink).

Characterizing the elimination of pharmaceuticals and biocides during wastewater treatment remains a challenge for researchers with respect to: (i) the number of substances produced, marketed and consumed worldwide; and (ii) sampling and analysis for XTCs in wastewater. Modelling the fate of trace chemicals during wastewater treatment can help overcoming these challenges and provide for additional benefits (e.g., support to decision-making).

Nevertheless, robust fate models require a system-level comprehension of XTC removal mechanisms. A lack of mechanistic understanding can still be identified in a number of areas, concerning XTCs fate in WWTPs:

- Most of pharmaceuticals and biocides have one or more ionizable groups (Manallack, 2009; Franco et al. 2010) and can thus be present in charged form. XTC ionization is likely to influence chemical partitioning to solid matrices (Franco and Trapp, 2008; Franco et al., 2009) and biological degradation (Gulde et al., 2014).
- XTCs are present at trace levels in wastewater, i.e. in ng L^{-1} up to low µg L^{-1} concentration range, and typically cannot be utilized as growth substrate by microbial populations. Their degradation may thus occur via secondary substrate utilization and cometabolism (Alexander, 1985; Rittmann, 1992; Nyholm et al., 1996; Plósz et al., 2010a, 2012; Fischer and Majewski, 2014) or mixed substrate utilization strategies (Egli, 2010; Tan et al., 2013).
- Pharmaceuticals can be formed during wastewater treatment via e.g., the degradation of respective excreted conjugated metabolites. These mechanisms, known under the general term 'retransformation', have been characterized for few substances (Ternes, 1998; Joss et al., 2004; Gomes et al., 2009; Plósz et al., 2010a, 2012). Retransformation processes have been described also in sewer systems (Jelic et al., 2015), influencing the loading of pharmaceuticals to WWTPs. Nevertheless, the impact of retransformation on the elimination of pharmaceuticals in WWTPs has not been fully clarified yet.

Eventually, a major and relevant endpoint of XTC emissions from WWTPs is represented by agricultural systems (Fig. 1.1). Due to the incomplete elimination during wastewater treatment, XTCs persist in WWTP effluents and sewage sludge. Reuse of municipal biosolids and treated wastewater for agricultural purposes (fertilization, irrigation) is common practice worldwide (Ternes et al., 2007; Sabourin et al., 2012; Sato et al., 2013; Prosser et al., 2014a), often encouraged at regulatory level (e.g., 86/278/EEC Directive in the European Union). Emissions also occur via irrigation with freshwater that contains XTC residues from WWTP effluents (Calderón-Preciado et al., 2011). Reuse of treated (and untreated) sewage for crop irrigation leads to higher crop yields and is increasingly exploited in arid and semiarid areas as a result of water shortages (Calderón-Preciado et al., 2011; Goldstein et al., 2014; Dalkmann et al., 2014). Besides release from WWTPs, amendment

with manure containing veterinary pharmaceuticals also results in emissions to agricultural soils (Boxall et al., 2003). Overall, these practices may determine uptake and subsequent accumulation of XTCs in food crops, eventually leading to dietary intake. Information on human exposure to XTCs via food crop consumption is scarce, and effects for human health are mostly unclear (Legind and Trapp, 2009; Prosser and Sibley, 2015; Malchi et al., 2015). Preliminary evidences showed that ingestion of pharmaceuticals via contaminated food crops often exceeds intake via other environmental sources, such as groundwater or fish consumption.

1.2 Objectives of the thesis

The overall objective of this PhD thesis was to develop or innovatively apply suitable mathematic modelling tools to describe the fate of XTCs (Fig. 1.2): (i) at fate process scale, combining experimental observations and model-based assessment (**Paper I, Paper II**); (ii) in full-scale WWTPs, using a model-based assessment methodology to evaluate the significance of factors influencing XTC elimination during biological treatment (**Paper III**); (iii) at regional scale in integrated WWTP-agricultural systems (**Paper IV**). Modelling tools used as reference in this study include the Activated Sludge Modelling framework for Xenobiotics (ASM-X; Plósz et al., 2010a, 2012, 2013) (**Paper I, II, III**), the multimedia model Activity SimpleTreat (Franco et al., 2011) and the dynamic soil-plant uptake model (Rein et al., 2011; Legind et al., 2012; Trapp and Eggen, 2013; Prosser et al., 2014b) (**Paper IV**).

The specific objectives of this PhD study were:

i. To evaluate experimentally the influence of pH and iron salt dosing, used for chemical phosphorus removal, on the sorption of ionizable XTCs onto activated sludge (**Paper I**)

ii. To assess and/or simulate the impact of XTC ionization on sorption onto activated sludge (**Paper I**), full-scale WWTP elimination and plant uptake (**Paper IV**) and to evaluate the associated implications for fate modelling

iii. To experimentally characterize the elimination of XTCs in pre-denitrifying laboratory-scale moving bed biofilm reactors (MBBRs) and to provide a model-based evaluation of the influence of primary substrate loading on XTC biotransformation (**Paper II**);

iv. To evaluate and predict—using a model-based generalization methodology (Plósz et al., 2012) and literature data—the impact of selected factors

(XTC retransformation, in-sewer reactions and solid residence time—SRT) on the full-scale XTC elimination during biological wastewater treatment (**Paper III**);

v. To develop and test a modelling tool for fate prediction of XTCs from human consumption and excretion to plant uptake in agricultural systems, evaluating the impact of agricultural sludge reuse and irrigation on XTC accumulation in food crops (**Paper IV**). The modelling tool was developed to further estimate potential risk from human exposure to XTCs via food crop intake and thus to support pre-screening and priority setting of potentially hazardous XTCs.

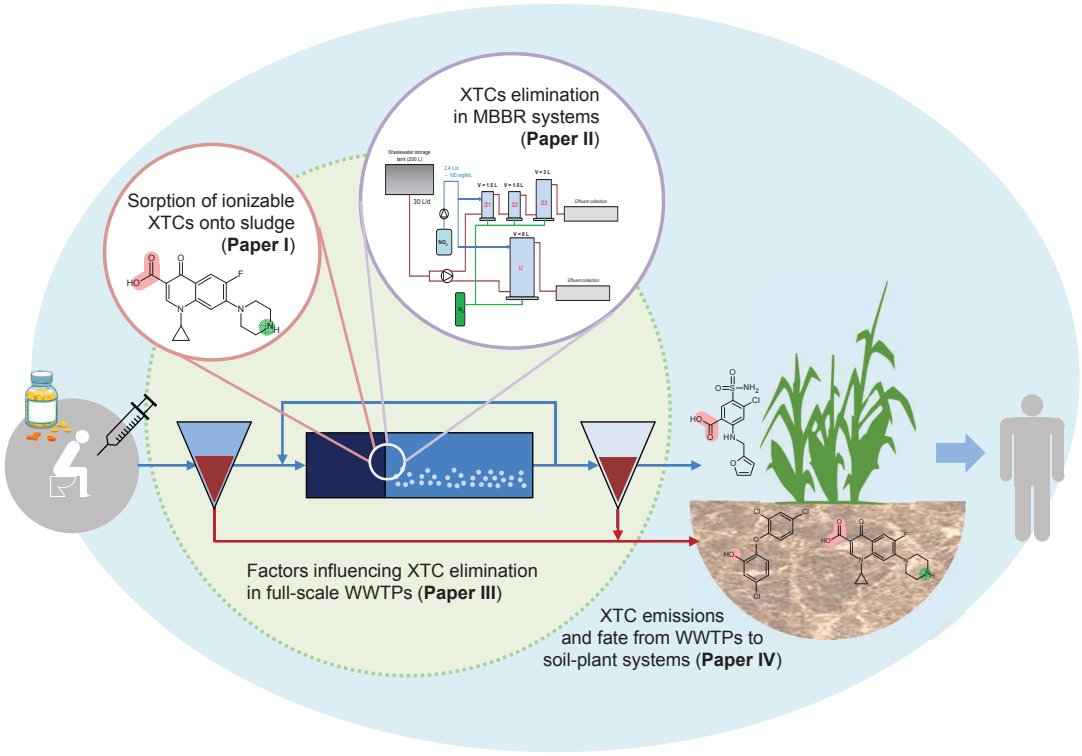


Figure 1.2. Schematic presentation of the model-based research activities conducted in this PhD thesis: (i) at fate process scale (Paper I, II), combined to experimental investigation; (ii) in full-scale WWTPs (Paper III); and (iii) at regional scale, in integrated WWTP-agricultural systems (Paper IV).

Our investigations focused on commonly used biocides (triclosan) and pharmaceuticals, i.e. antibiotics (ciprofloxacin, sulfonamides and macrolides), anti-inflammatory drugs (diclofenac, ibuprofen), beta-blockers (atenolol, metoprolol), diuretics (furosemide), antidepressants (venlafaxine) and X-ray contrast media (iohexol).

In this PhD thesis, the acronym XTC (xenobiotic trace chemicals) will be used from now on as common term for pharmaceuticals and biocides and their excreted metabolites.

2 Fate of XTCs during wastewater treatment

2.1 Wastewater treatment configurations

Significant amounts of pharmaceuticals are released from households, hospitals and other facilities and eventually reach municipal wastewater treatment plants (WWTPs). WWTPs typically employ several wastewater treatment steps, from the removal of course solids to final polishing steps. This PhD thesis focused on the model-based and experimental assessment of XTC removal during secondary treatment only (**Paper I, II, III**) and during primary and secondary treatment (**Paper IV**).

Primary treatment typically consists of a sedimentation step (primary clarification) for the removal of influent total suspended solids (TSS). Chemical dosing (coagulants/flocculants or iron/aluminium salts) can be performed to achieve increased solid removal efficiencies and pre-precipitation of influent phosphorus (Tchobanoglous et al., 2014; Wentzel et al., 2008).

Secondary treatment includes a wide range of engineered biological systems for the removal of organic matter (measured as BOD/COD, biological/chemical oxygen demand) and nutrients (nitrogen, phosphorus). Common treatment configurations investigated in this PhD thesis (through experimental or model-based assessment or as a result of literature review) are presented in Figure 3. A general distinction is considered between suspended growth (activated sludge-based) and attached growth (biofilm-based) systems. In the former, activated sludge bioreactors are conventionally coupled to secondary sedimentation to separate and recirculate sludge biomass from treated effluent (Fig. 3a–c). Alternatively, submerged membranes are employed for biomass retention in bioreactors (membrane bioreactors—MBR, Fig. 3e). Solid residence time (SRT) or sludge age is the key operating parameter for suspended growth systems, allowing for the selection of a desired microbial function. Activated sludge systems for COD removal (SRT $\leq$ 5 d; Ekama and Wentzel, 2008) consist of single-stage aeration. Biological nutrient removal (BNR) requires higher SRTs ($\geq$ 8–10 d; Ekama and Wentzel, 2008) for the growth of nitrifying bacteria. Most common examples of BNR systems are: (i) the Ludzack-Ettinger configuration with pre-denitrification (using influent COD as electron donor), nitrification and mixed liquor recirculation (Fig. 3b,e); (ii) the A2O (anaerobic/anox/aerobic) configuration for

enhanced biological phosphorus removal (EBPR; Fig. 3c). In these systems, phosphorus removal can be chemically achieved/enhanced via salt dosing (co- or post-precipitation; Wentzel et al., 2008).

Biofilm systems are characterized by a number of advantages over conventional activated sludge systems, namely prolonged biomass retention and reduced biomass separation requirements. Among these systems, increasing attention has been posed on moving bed biofilm reactors (MBBRs) (Ødegaard et al., 1994). In MBBRs, biomass is immobilized on suspended plastic carriers (Fig. 3d, detail), which undergo mixing by aeration or mechanical stirring. Common full-scale applications of MBBRs include nitrogen removal in BNR systems and effluent polishing (e.g., post-denitrification) (Rusten, 2005; Ødegaard, 2006). A combined configuration is presented in Fig. 3d.

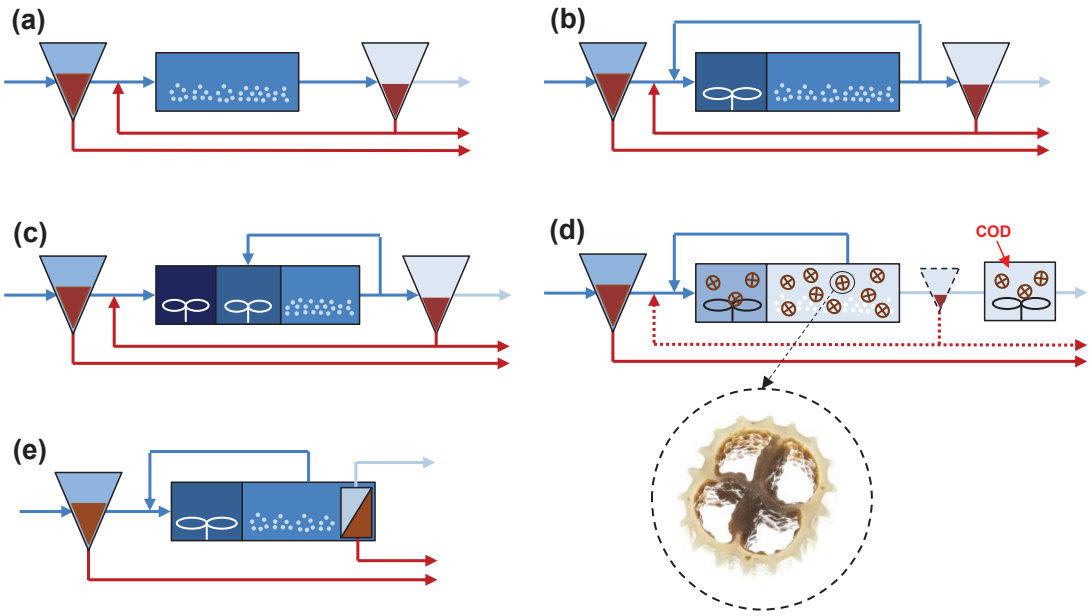


Figure 2.1. Schematic representation of typical full-scale WWTP layouts employing primary and secondary treatment. Typical secondary treatment configurations assessed in this PhD thesis (experimentally, through modelling exercise or as a result of literature review) include: (a) aerated activated sludge system for COD removal (and nitrification) with secondary sedimentation; (b) pre-denitrifying Ludzack-Ettinger configuration with secondary sedimentation; (c) A^2O configuration for enhanced biological phosphorus removal; (d) moving bed biofilm reactors (MBBR), employing plastic biofilm carriers (detail: Anox-Kaldnes K1 carrier), in Ludzack-Ettinger configuration with post-denitrification; (e) pre-denitrifying Ludzack-Ettinger configuration with nitrifying membrane bioreactor (MBR).

2.2 Fate processes

Elimination of influent XTCs during wastewater treatment is the combined result of a number of processes. Major processes responsible for XTC elimination will be described in the following sub-sections.

2.2.1 Biotransformation/biodegradation

Biotransformation and biodegradation are equivalent terms used to describe microbially-mediated transformations of organic xenobiotics to other chemical products. Subsequent steps of microbial transformation eventually lead to the complete mineralization of xenobiotics to inorganic carbon, nitrogen and water (Schwarzenbach et al., 2003). Mineralization of XTCs rarely occurs in wastewater systems, with accumulation of multiple transformation products (see, e.g., Khunjar et al., 2011; Rubirola et al., 2014). Biotransformation and biodegradation are thus most appropriate to describe the biotic elimination of XTCs, and will be used within this PhD thesis. In this section, we will present different mechanisms of biotransformation and factors influencing this process.

Biotransformation of XTCs in ng L^{-1} to µg L^{-1} concentrations does not support biomass growth and energy requirements (Alexander, 1985; Rittmann, 1992; Nyholm et al., 1996) and thus results from microbial cometabolism (i) in growing cells in the presence of growth/energy substrate and (ii) in resting cells in the absence of growth/energy substrate (Criddle, 1993). Case (ii) describes, e.g., cell maintenance via endogenous respiration. Cometabolic biotransformation further stems from the lack of specific enzymes catalysing defined transformation processes (Grady et al., 2011), being thus 'fortuitously' (Fischer and Majewski, 2014) catalysed by broad spectrum enzymes in autotrophic and heterotrophic populations (Helbling et al., 2010; Khunjar et al., 2011; Helbling et al., 2012).

For a number of organic xenobiotics, biotransformation was described as a metabolic process supporting the growth of specialist degrader populations (detergent builders: Siegrist et al., 1989; endocrine disrupters: Lindblom et al., 2009). More recently, this hypothesis was tested by associating biodiversity (Johnson et al., 2015) and taxonomic abundance (Helbling et al., 2015) to XTC biotransformation rates. Associations were demonstrated only for a limited number of XTCs (ranitidine, venlafaxine), being degraded via uncommon transformation pathways. It was suggested that specialist degraders are responsible only for a limited number of rarely occurring transformations (Johnson et al., 2015; Helbling et al., 2015), whereas less rare transfor-

mations are probably catalysed, via cometabolism, by commonly present non-specific enzymes.

Consequently, primary growth and energy substrates play a significant role in the biotransformation of XTCs. The presence of growth substrate, effectively supporting biomass growth, was shown to be beneficial for XTC biotransformation in batch (Plósz et al., 2012) and continuous-flow experiments (Tan et al., 2013; Su et al., 2015). Nevertheless, growth substrate inhibition of XTC biotransformation was also reported (Joss et al., 2004; Plósz et al., 2010a; Su et al., 2015; Mazioti et al., 2015), resulting from, e.g., competition for non-specific enzymes.

Conversely, strongly limiting growth substrate availability was hypothesized to improve XTC biotransformation due to (i) reduced competitive inhibition effects; and/or (ii) expansion of biomass metabolic capabilities. Under oligotrophic conditions, microbial metabolism relies on the utilization of multiple substrates at low concentration (mixed substrate growth; Egli, 2010). Through broad catabolic enzyme expression, bacteria can utilize organic substances not typically used as growth substrates ('metabolic expansion'; Ihssen and Egli, 2005; Egli, 2010). Adaptation to growth substrate limitation may lead to the selection of K-strategist populations (Koh et al., 2009). Under prolonged biomass starvation, enhanced biotransformation rate of the estrogen E1 was observed and attributed to mixed substrate utilization by heterotrophic bacteria (Tan et al., 2013). Improved biotransformation by biomass exposed to limiting substrate availability was further observed for estrogens (Koh et al., 2009; Ziels et al., 2014; Petrie et al., 2014) and pharmaceuticals (Falås et al., 2013). Even under strongly limiting conditions, the availability of alternative substrates can be beneficial for XTC biotransformation by supporting catabolic enzyme induction (Egli, 2010). The type of available organic substrate can thus be influential. Increased biotransformation rate for E1 was found in the presence of recalcitrant organic substrate—namely products of bacterial decay and lysis—arising from prolonged biomass starvation (Tan et al., 2015).

Overall, there is limited information on the importance of growth substrate availability for the biological transformation of XTCs. Furthermore, most of available evidences refer to observations under aerobic conditions. In **Paper II**, we assessed XTC biotransformation by pre-denitrifying biomass exposed to different growth substrate availability and loading conditions. By testing the hypotheses presented above, we aimed at discriminating whether (i)

growth substrate supports XTC biotransformation as a result of cometabolism; or (ii) strongly limiting substrate availability is beneficial for XTC biotransformation.

Among other operational factors, SRT was hypothesized to influence XTC biotransformation capacity of suspended and attached biomass. Improved XTC biotransformation at increasing SRT was considered the result of increased microbial diversity (e.g., enrichment of slowly growing bacteria) or of metabolic capabilities (e.g., mixed substrate growth) (Ternes et al., 2004b). Specifically, the positive influence of SRT on XTC elimination was considered to occur: (i) in the presence of nitrifiers (SRT > 5 d in fully aerobic systems and > 8–10 d in aerobic-anoxic systems; Ekama and Wentzel, 2008); and (ii) in systems with enhanced physical biomass retention, thus operating at extended SRTs (e.g., MBRs, MBBRs). Critical SRTs have been accordingly identified with respect to the first (10 d; Clara et al., 2005a,b) and the second case (e.g., 20 d for diclofenac; Plósz et al., 2012), at which increased XTC elimination was shown due to a step improvement in biotransformation kinetics. To date, however, no generalization on the impact of SRT could be made.

XTC can be co-metabolized by ammonia oxidizing bacteria (AOB) via the broad-spectrum enzyme ammonia monooxygenase (AMO) (Vader et al., 2000; Shi et al., 2004; Yi and Harper, 2007b). Enhanced XTC biotransformation in active and resting nitrifiying cultures was accordingly observed for estrogens and pharmaceuticals (e.g., Vader et al., 2000; Batt et al., 2006; Forrez et al., 2008; Khunjar et al., 2011; Sathyamoorthy et al., 2013). However, this could not be confirmed for all the XTCs assessed (Khunjar et al., 2011; Sathyamoorthy et al., 2013). Conversely, the contribution of AOB to XTC biotransformation has been disputed (Gaulke et al., 2008) or deemed limited (e.g., McAdam et al., 2010; Majewsky et al., 2011a; Bagnall et al., 2012; Helbling et al. 2012; Petrie et al., 2014a) as compared to heterotrophic bacteria.

Operation at extended SRT in MBRs and biofilm reactors (e.g., MBBRs) may enhance XTC biotransformation due to (Joss et al., 2006b; Weiss and Reemtsma, 2008; Oulton et al., 2009; Le-Minh et al., 2010; Vuono et al., 2014): (i) improved adaptation to trace chemicals; (ii) enrichment of slow growing organisms, capable of utilizing XTCs as a resource; (iii) reduced substrate availability from low food-to-microorganism (F/M) ratio, inducing the utilization of low concentration XTCs as substrates (mixed substrate me-

tabolism: Egli, 2010). Improvement of XTC biotransformation and elimination in MBRs, as compared to conventional activated sludge, was accordingly reported for, e.g., estrogens (Joss et al., 2004, 2006b) and few anti-inflammatory pharmaceuticals (Joss et al., 2006b; Bernhard et al., 2006; Radjenovic et al., 2009). Negligible differences between MBRs and conventional activated sludge systems were frequently observed (Clara et al., 2005; Joss et al., 2005; Bernhard et al., 2006; Abegglen et al., 2009; Sui et al., 2011). More recently, biotransformation in MBBRs under aerobic conditions was found to occur at higher rates than in activated sludge for a number of pharmaceuticals (Falås et al., 2012, 2013), though not being confirmed for all tested XTCs.

To date, evidences on the influence of SRT on biotransformation could not be generalized for all XTCs investigated, leading to the conclusion that the impact of such operational parameter may be chemical-specific. In this PhD thesis, we aimed at increasing the understanding in this matter by assessing the biological transformation of a number of XTCs in pre-denitrifying MBBRs (**Paper II**). Furthermore, we proposed and successfully tested a methodology to assess the impact of SRT in the full-scale removal of XTCs by comparing model predictions and literature data (**Paper III**).

2.2.2 Sorption/desorption

Partitioning of XTCs to solid matrices in wastewater (e.g., suspended solids, suspended or attached biomass) is a physico-chemical process involving two reverse mechanisms, i.e. sorption from aqueous to solid phase and desorption from solid to aqueous phase (Joss et al., 2006b). As an equilibrium process, solid-liquid partitioning is considered completed when the rate of sorption equals the rate of desorption, i.e. there is not net change in the XTC concentrations in solid and aqueous phase. For each XTC at equilibrium, the ratio between these two concentrations is fixed, and the extent of sorption can be described by the solid-liquid partition coefficient K_d (L $gTSS^{-1}$; Eq. 2.1):

$$K_d = X_{SL,eq} / C_{LI,eq} = C_{SL,eq} / (C_{LI,eq} X_{TSS}) \quad (2.1)$$

where $X_{SL,eq}$ (µg $gTSS^{-1}$) and $C_{SL,eq}$ (µg L^{-1}) denote the concentration in solid phase at equilibrium per gram sorbent and per litre of bulk solution, respectively, C_{LI} (µg L^{-1}) the aqueous concentration. X_{TSS} (gTSS L^{-1}) is most frequently used to indicate the concentration of sorbent in solution. Eq. 1 represents a simplified (linear) case of the empirical Freundlich equation (Freundlich, 1909; Eq. 2.2):

$$X_{SL,eq} = C_{SL,eq} / X_{TSS} = K_f \left(C_{LI,eq}\right)^n \quad (2.2)$$

where K_f denotes the Freundlich coefficient ($\mu g^{1-n}\ L^n\ g^{-1}$) and n the linearity parameter (-). This equation allows accounting for saturation (n<1) or synergistic (n>1) partitioning effects at high XTC concentrations. Partitioning can be considered as an affinity process between two organic phase (XTCs and organic matter in solids), and can be further described the by the partition coefficient normalized to organic carbon, K_{oc} (Karickhoff et al., 1979; Eq. 2.3):

$$K_{oc} = K_d / f_{oc} \quad (2.3)$$

where f_{oc} denotes the organic carbon fraction in the sorbent (e.g., gOC $gTSS^{-1}$).

Sorption/desorption equilibrium is assumed to be reached instantaneously, defining partitioning as a non-rate-limiting process during wastewater treatment. Experimental observations confirmed this assumption, showing that equilibrium can be reached within 0.5–1 h (Ternes et al., 2004a; Andersen et al., 2005; Yi and Harper, 2007; Khunjar and Love, 2011; Plósz et al., 2013). Particulate matter represents a rather heterogeneous sorbent for XTCs in wastewater. Partitioning can in fact occur—to different extents—onto hydrolysable organic matter, colloidal organic carbon, exopolymeric substances (EPSs) and cell surfaces (Siegrist et al., 2003; Holbrook et al., 2004; Barret et al., 2010a,b; Khunjar and Love, 2011; Delgadillo-Mirquez et al., 2011). In addition, sorption onto dissolved organic matter was found significant for hydrophobic chemicals (e.g., PAHs) (Barret et al., 2010a,b; Delgadillo-Mirquez et al., 2011). In wastewater systems, XTCs undergo solid-liquid partitioning in raw sewage, reaching equilibrium during in-sewer transport, and during secondary treatment, onto suspended or attached biomass (Ternes et al., 2004b; Senta et al., 2013; Petrie et al., 2015). Removal of sorbed XTCs from sewage mostly occurs in primary settlers, via sedimentation of raw influent solids as primary sludge, and secondary settlers, with sedimentation and subsequent wastage of (a fraction of) activated sludge. The relative importance of these two processes may be substantially different, due to primary and secondary sludge composition and physico-chemical characteristics (Ternes et al., 2004b).

Traditionally, partitioning has been associated to hydrophobicity of chemicals, and K_{oc} predictions based on K_{ow} (octanol-water partition coefficient of the neutral species) or D (pH-dependent distribution coefficient) have been

established (e.g., Sablijc et al., 1995). This approach was proved insufficient in predicting XTC sorption onto activated sludge (Ternes et al., 2004a; Hörsing et al., 2011; Stevens-Garmon et al., 2011; Hyland et al., 2012; Sathyamoorthy and Ramsburg, 2013). Other major partitioning mechanisms have been accordingly identified (MacKay and Vasudevan, 2012), namely electrostatic interactions (e.g., Coulombic attraction or cation exchange) and surface complexation. In wastewater systems, pH and ionic strength of the aqueous solution can thus influence sorption by (i) changing the surface chemistry of the sorbent (Wang et al., 2000); and (ii) most importantly, changing the activity of the ionizable sorbate or leading to the formation of complexes (Trapp et al., 2010; MacKay and Vasudevan, 2012). Where detailed evidences on the effect of pH on the partitioning of ionizable XTCs have been obtained for soil matrices (Franco and Trapp, 2008; Franco et al., 2009; see 2.2.4), little information is available for sorbents in wastewater systems (e.g., sludge matrices). In this PhD thesis, we aimed at providing insights on the influence of pH and ionic strength on the partitioning of an ionizable antibiotic (the zwitterionic ciprofloxacin) onto activated sludge (**Paper I**).

Chemicals can be entrapped to remote regions of the solid matrix, a process also known as sequestration or slow sorption. Sequestered amounts cannot be considered in equilibrium with the aqueous phase, thus being potentially not bioavailable (Schwarzenbach et al., 2003). The significance of sequestration was earlier shown in soils and sediments (Pignatello and Xing, 1996; Alexander, 2000). Experimental (Kim et al., 2005; Yi and Harper, 2007a; Wu et al., 2009) and model-based (Plósz et al., 2012) observations in sewage sludge showed that XTC sequestration may also be relevant to wastewater systems. Sorbed XTCs may thus include a sequestered fraction undergoing extremely slow or no desorption (thus exhibiting 'sorption hysteresis'; Yi and Harper, 2007a).

2.2.3 Retransformation

The increase of aqueous concentrations, corresponding to the formation of parent pharmaceuticals, has been observed during laboratory-scale experiments and in full-scale WWTPs (resulting in 'negative' removal efficiencies; Stadler et al., 2012). Different processes may be responsible for such observations, and have been thus grouped under the general term 'retransformation'. The influence of retransformation on the full-scale elimination of the antibiotic sulfamethoxazole was assessed in **Paper III**. Here we present a detailed assessment of the main observed retransformation mechanisms (also summarized in Fig. 2.2).

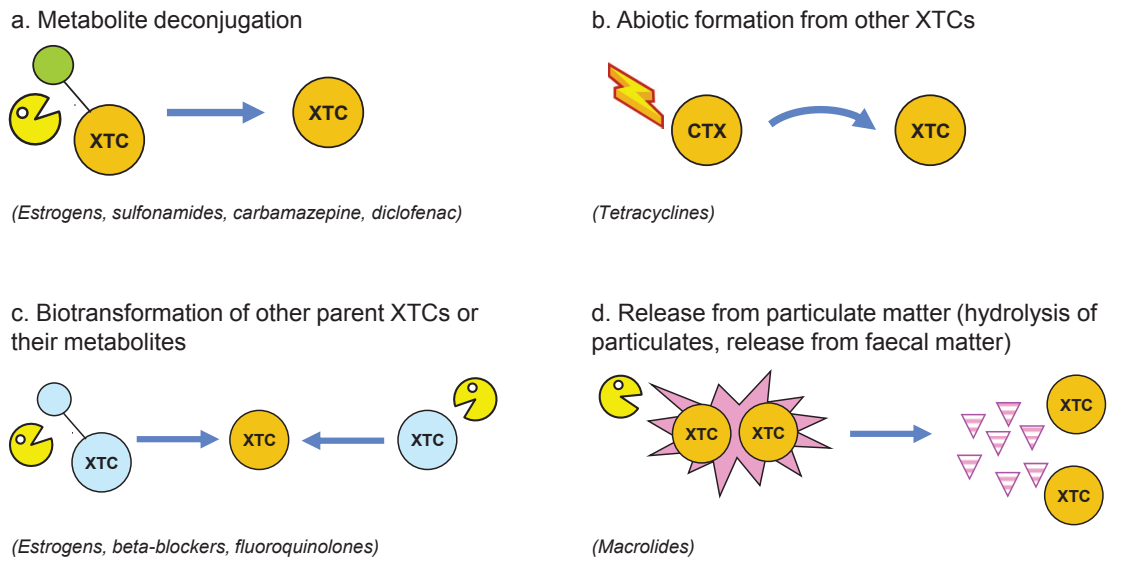


Figure 2.2. Summary of the main mechanisms leading to the formation of parent XTCs in aqueous phase—indicated by the orange circle (adapted from Plósz et al., 2013b). XTCs in parentheses refer to chemicals, for which each different mechanism was observed and/or hypothesized. A detailed description of the different mechanisms is provided in the paragraphs below.

Metabolite deconjugation

Following administration, XTCs are metabolized via: (i) functionalization reactions (e.g., oxidation, hydroxylation, reduction, mostly catalysed by CYP450 enzymes); and (ii) conjugation reactions (e.g., glucuronidation, sulfation, acetylation) to facilitate the urinary excretion of metabolites (Zhang et al., 2008). These processes have been observed in humans, animals (Grafström et al., 1979) and plants (Komossa et al., 1995). Formerly known as Phase I and Phase II reactions, respectively, these two types of processes can occur independently of each other, resulting e.g., in the direct conjugation of parent XTCs (Gruengerich, 2008; Testa et al., 2012). Parent XTCs and metabolites are eventually excreted with urine (the largest fraction) and bile/faeces. Excreted conjugates of the parent XTCs (approximately 22% of first-step human metabolites; Testa et al., 2012) may retransformation back to the parent form following cleavage of the conjugated moiety—also known as deconjugation. In wastewater and water systems, biological deconjugation was hypothesized or observed for several XTCs: (i) natural and synthetic estrogens (Ternes et al., 1999; Andersen et al., 2003; Joss et al., 2004; Gomes et al., 2009); (ii) carbamazepine (Ternes, 1998; Joss et al., 2005; Vieno et al., 2007; Plósz et al., 2012; Bahlmann et al., 2014) and its functionalized metabolites (Miao et al., 2005; Bahlmann et al., 2014; Gurke et al., 2015); (iii)

diclofenac (Lee et al., 2012; Plósz et al., 2012; Vieno and Sillanpää, 2014); (iv) sulfonamide antibiotics (Göbel et al., 2005, 2007; Joss et al., 2006a; Radke et al., 2009; Plósz et al., 2010a; García-Galán et al., 2012a; Falås et al., 2013; Senta et al., 2013; Stadler et al., 2015); (v) ibuprofen (de Graaff et al., 2011). Microbial deconjugation is typically a fast process (Joss et al., 2006), resulting in the almost complete elimination of conjugates in full-scale WWTP (Göbel et al., 2005, 2007; Stadler et al., 2015). Nevertheless, relative persistence of certain conjugates (e.g., N-glucuronides) has been hypothesized (Bahlmann et al., 2014). Although the significance of deconjugation during wastewater treatment has been acknowledged, detailed empirical evidences are still scarce—being limited to estrogens—mainly due to analytical challenges (García-Galán et al., 2008; Celiz et al., 2009; Griffith et al., 2014; Brown and Wong, 2015).

Abiotic formation

Few examples of abiotic retransformation of parent XTCs have been reported. Tetracycline antibiotics are excreted in unchanged and epimerized form (Sengeløv et al., 2003; Agwuh and MacGowan, 2006). In aqueous media, unchanged tetracyclines can be further epimerized (McCormick et al., 1957; Remmers et al., 1963). Reverse epimerization—with formation of unchanged tetracycline—was observed in activated sludge and soil under neutral to alkaline conditions and in the presence of divalent cations (Halling-Sørensen et al., 2002).

Formation from other XTCs

Parent XTCs and/or metabolites can be the transformation products of other XTCs during biological wastewater treatment. The most known example is the formation of the estrogen E1 (estrone) from E2 (17β-estradiol) observed (Ternes et al., 1999; Joss et al., 2004; Urase and Kikuta, 2005) and modelled (Joss et al., 2004; Urase and Kikuta, 2005) in activated sludge. The reverse process was observed under anaerobic conditions (Joss et al., 2004). Analogous processes include the formation of (i) atenolol acid (a metabolite of metoprolol) from atenolol in activated sludge (Radjenovic et al., 2008); (ii) ciprofloxacin from fungal degradation of enrofloxacin (Wetzstein et al., 2006). Notably, commercial XTCs can be formed from the human metabolism of other substances (e.g., diazepam metabolized to oxazepam; Kovalova et al., 2012), thus generating additional emissions to WWTPs without being directly consumed.

Release from particulate matter

Formation of XTCs in aqueous phase had been eventually attributed to the release of amounts present in solid phase via a number of hypothesized mechanisms: (i) release from faecal matter e.g., of macrolides (Göbel et al., 2007), justifying aqueous concentration increases from WWTP influent to effluent (Terzic et al., 2008; Gao et al., 2012; Oertel et al., 2014); (ii) biotic exocellular hydrolysis of particulate, colloidal and dissolved organic matter (Larsen and Harremoës, 1994; Guellil et al., 2001), on which XTCs are sorbed (Holbrook et al., 2004; Barret et al., 2010a,b; Delgadillo-Mirquez et al., 2011); and (iii) desorption due to changes in wastewater conditions (e.g., pH).

2.2.4 Ionization

A significant fraction of marketed XTCs is ionizable (Manallack, 2009; Franco et al., 2010) and can be present in neutral and/or ionized form at environmental pH conditions. Ionization is as an instantaneous process in aqueous solution and fractions of neutral and ionized species (anionic, cationic, zwitterionic) at equilibrium are expressed as a function of pH and ionization constants (pK_a) using Henderson-Hasselbach equations. For monovalent ionics (acids: α = 1; bases: α = -1), ionic fractions are calculated according to (Eq. 2.4–2.5):

$$\phi_n = 1/\left(1+10^{\alpha(pH-pK_a)}\right) \tag{2.4}$$

$$\phi_i = 1-\phi_n \tag{2.5}$$

where the subscripts 'n' and 'i' denote neutral and ionic (anionic or cationic) species, respectively. XTCs in relevant categories (e.g., fluoroquinolone and tetracycline antibiotics) are zwitterionic and undergo internal proton migration, with coexistence of two oppositely charged groups in an overall neutral molecule. The latter speciation coexists with the truly neutral (uncharged) configuration, without internal proton migration (Pagliara et al., 1997). Zwitterionic XTCs can thus speciate as anions (-), cations (+), zwitterions (±) and in uncharged form (0), and the respective fractions are determined from (Eq. 2.6–2.10):

$$\phi_n = 1/\left(1+10^{(pH-pK_{a,2})}+10^{(pK_{a,1}-pH)}\right) \tag{2.6}$$

$$\phi_+ = \phi_n\left(10^{(pK_{a,1}-pH)}\right) \tag{2.7}$$

$$\phi_- = \phi_n\left(10^{(pH-pK_{a,2})}\right) \tag{2.8}$$

$$\phi_n = \phi_{\pm} + \phi_0 \tag{2.9}$$

$$K_Z = \frac{\phi_{\pm}}{\phi_0} \tag{2.10}$$

where $pK_{a,1}$ and $pK_{a,2}$ denote the acidic and the basic ionization constant, respectively. The ratio between zwitterionic and uncharged fraction (which combined represent the neutral configuration) is known as constant of tautomeric equilibrium K_Z (Eq. 2.10; Pagliara et al., 1997).

Ionization of XTCs has implications on other physico-chemical and biological fate processes. When occurring via non-hydrophobicity-related mechanisms (2.2.2), solid-liquid partitioning is influenced by the charge of sorbent and sorbate (Trapp et al., 2010). Sorbents (soil, activated sludge) typically exhibit negatively charged surfaces (organic acidic groups, clay minerals) at environmental pH (e.g., Wang et al., 2000; Delle Site, 2001; MacKay and Vasudevan, 2012), determining e.g., limited sorption potential of anionic species due to repulsion effects (Carrasquillo et al., 2008; Franco et al., 2009; Tülp et al., 2009; Vasudevan et al., 2009). Furthermore, uptake of XTCs in cells is significantly slower for ionized than for neutral species, influencing XTC accumulation in plants (Trapp, 2004), bioaccumulation and toxicity in algae and fish (Rendal et al., 2011) and biotransformation in activated sludge systems (Gulde et al., 2014). Overall, the extent of the impact by XTC ionization is determined by the pH conditions, at which these processes occur.

2.2.5 Other processes

A number of other processes have been traditionally considered for the removal of organic xenobiotics in wastewater treatment systems (see, e.g., Vezzaro et al., 2014), including (i) volatilization and stripping; (ii) photolysis; and (iii) chemical hydrolysis. These processes may be of limited significance for the removal of XTCs assessed in this PhD thesis. Elimination from the aqueous phase via volatilization and/or stripping (induced, e.g., by aeration in activated sludge reactors) is considered negligible, as expected by the extremely low volatility of XTCs (dimensionless air-water partition coefficient $K_{aw} < 4 \cdot 10^{-6}$; Trapp and Harland, 1995) High turbidity of raw sewage and mixed sludge liquor is likely to hinder photolysis of XTCs in conventional WWTPs (Michael et al., 2013). Chemical hydrolysis of pharmaceuticals was also shown negligible in activated sludge (Li and Zhang, 2010). Nevertheless, this process was shown to be responsible for the removal of illicit drugs in raw sewage (Senta et al., 2014; Ramin et al., in preparation). Simul-

taneous evaluation of the listed processes in laboratory-scale abiotic degradation experiments (Helbling et al., 2010; Gulde et al., 2014) showed their overall limited relevance for the elimination of tested pharmaceuticals.

3 Modelling the fate of XTCs during (and beyond) wastewater treatment

Wastewater treatment systems receive emissions of a wide range of synthetic organic chemicals, motivating a three-decade tradition of fate models. Early attempts focused, among others, on the fate of down-the-drain chemicals in consumer products (e.g., surfactants; Siegrist et al., 1989). Recent attention has been addressed to pharmaceuticals and biocides (XTCs) as a result of their widespread consumption and of the acknowledged environmental risks associated to them.

In agreement with the former regulation on the risk assessment of pharmaceuticals (EMEA, 2006), simplified approaches have been often adopted in regional fate studies. Removal efficiency in WWTPs has been in fact neglected (Stuer-Lauridsen et al., 2000; Besse and Garric, 2008) or considered as a fixed (Grung et al., 2009; Morasch et al., 2010) or variable (Johnson and Williams, 2004; Ort et al., 2009; Johnson et al., 2013) sink term based on full-scale measurements.

Research in the field of XTC fate modelling generally faced two major challenges: (i) understand the mechanisms of XTC elimination during (biological) wastewater treatment; (ii) provide for reliable predictions (for, e.g., risk assessment studies) with limited data requirement. Accordingly, models developed and tested for XTC fate prediction can be distinguished in two major categories: multimedia (fugacity and activity) models and concentration-based models. Multimedia models require limited input and are typically used in generic fate and exposure assessments (Keller, 2006). Conversely, concentration-based models were used for fate predictions in selected laboratory- and/or full-scale systems (also at catchment level), often implemented as extension of Activated Sludge Models (ASMs; Henze et al., 2000) and. In both cases, modelling exercises have primarily focused on the elimination during primary and secondary wastewater treatment. Eventually, the use of either model types aimed at predicting (i) XTC removal efficiencies and fractions of XTC influent load removed via relevant processes (e.g., partitioning); and (ii) XTC concentrations at different stages (i.e., primary or final effluent) and in different compartments (wastewater, sludge) of wastewater treatment.

Relevant examples of these two types of model have been assessed and compared (Table 3.1) according to different criteria: (i) conditions assessed (steady-state or dynamic); (ii) fate processes included; (iii) scale of applica-

tion (laboratory, pilot and/or full scale); and (iv) XTC categories investigated. A brief presentation of the history and the main features of multimedia and concentration-based models is given in the following paragraphs.

3.1 Multimedia fugacity and activity models

3.1.1 Fugacity-based models

Multimedia models are generally defined as mathematical tools used for fate predictions of organic chemicals in systems subdivided in a number of distinct well-mixed compartments (or 'media') (Trapp and Matthies, 1998). Early examples of multimedia models ("Mackay models") were established to predict the distribution of organic contaminants in different media (air, water, soil, sediments) of an ideal environment section (the 'Unit World'; Mackay, 1979), under steady state conditions. Mackay models—and most of multimedia models in general—use fugacity as state variable to predict concentrations in environmental media. Fugacity (f; Lewis, 1901) is a thermodynamic property (partial pressure, Pa) describing the tendency for a chemical of escaping a certain phase. In analogy with thermodynamics, at equilibrium the fugacity in different compartments is equal (Mackay, 1979). Concentrations in different compartments are linearly correlated to the respective fugacities (Eq. 3.1):

$$C = fZ \tag{3.1}$$

where Z (mol m^{-3} Pa^{-1}) is defined as the fugacity capacity. Z quantifies the extent of partitioning to a different compartments, and can be calculated from typical distribution coefficients (e.g., from K_d for solids) (Mackay and Paterson, 1981).

Mackay and Paterson (1981, 1982) defined different modelling levels, depending on the conditions (steady-state or dynamic; equilibrium or non-equilibrium between compartments), on the type of systems assessed (open or closed) and on the occurrence of reactions (degradation) within media. To date, Mackay models have been applied and tested until level III (steady-state, open system, non-equilibrium, degradation), thus describing only systems in steady-state conditions. In case of non-equilibrium, chemical transfer processes (advection, partitioning) occur between compartments. Transfer rates are described using a first-order equation with respect to f (Eq. 3.2):

$$dm/dt = Df \tag{3.2}$$

where m is the mass of chemical (mol) and D (mol h^{-1}) is the transport coefficient (Mackay and Paterson, 1982). By calculating mass balances within each compartment (Eq. 3.2), fugacities (and thus concentrations, Eq. 3.1) in different compartments can be determined.

Multimedia models based on the fugacity approach have been considered for fate modelling in WWTPs. The most prominent example in this category is SimpleTreat, originally developed by Struijs et al. (1991) and progressively updated and revisited until v. 4.0 (Struijs, 2014; Fig. 3.1). SimpleTreat is a level III Mackay model simulating an ideal WWTP (10,000 PE) with primary settling (optional), aerated activated sludge reactor and secondary settler. Each reactor is subdivided in different media (air, water, suspended solids, sludge—'9 boxes') not in equilibrium with each other. Besides the raw sewage input to the WWTP (with XTC present both in water and suspended solids), processes simulated in the system include: (i) advection between different reactors; (ii) diffusion (i.e. partitioning) between different media (e.g., volatilization, sorption); and (iii) XTC biodegradation in aqueous phase or in aqueous and solid phase (depending on modeller's choice). SimpleTreat can predict the fractions of influent XTC load removed via different pathways (volatilization, sorption onto primary or secondary sludge, biodegradation) as well as XTC concentrations in sludge and final effluent.

Other examples of fugacity WWTP models include STP (Clark et al., 1995) and STP-EX (Seth et al., 2008). A simplified approach is used, assuming that media in each treatment stage are in equilibrium and thus reducing the number of state variables in the model (Table 3.1). SimpleTreat and STP were used to evaluate removal in alternative WWTP configurations to the default one, e.g. in activated sludge with intermittent operation (Fauser et al., 2003) or in aerated lagoons (Seth et al., 2008).

An important assumption of traditional multimedia fugacity models (SimpleTreat v. 3.1, STP, STP-EX) was that ionized species do not partition from water phase to adjacent media. Partition coefficients to organic carbon (K_{oc}) are accordingly predicted using K_{ow}-based regressions for non-polar hydrophobic organic chemicals (Sablijc et al., 1995). This assumption was shown to be rather unrealistic for solid-liquid partitioning. The necessity of accounting for partitioning of ionized species has been recently demonstrated, and alternative regressions were accordingly developed to predict K_{oc} of ionizable chemicals (Franco and Trapp, 2008; Franco et al., 2009, 2013a). More recent

SimpleTreat versions (3.2: Franco et al., 2013a,b; 4.0: Struijs, 2014) have been updated to include these regressions.

3.1.2 Activity-based models

An alternative approach to model the fate of ionizable XTCs in multimedia systems has been proposed by Trapp et al. (2010). In this approach, fugacity is replaced by the activity concept (Lewis, 1907). Activity a_t (mol m^{-3}) describes the apparent concentration of a chemical in non-ideal solutions, considering e.g. ionic strength effects (Franco and Trapp, 2010). In analogy with fugacity, the concentration in a compartment is linearly correlated to the chemical activity a_t (Eq. 3.3)

$$C = a_t B \tag{3.3}$$

where B is defined as the bulk activity capacity (–) analogous to the coefficient Z (Eq. 3.1). The novelty of this approach relies in the possibility of separately accounting for the activity of neutral (a_n) and ionized species (a_i), where the total activity at is equivalent to (Eq. 3.4):

$$a_t = a_n + \sum_i a_i \tag{3.4}$$

Neutral and ionic activities can be further determined from the respective ionic fractions ϕ_n and ϕ_i (Eqs. 3.5, 3.6):

$$a_n = a_t \phi_n \tag{3.5}$$

$$a_i = a_t \phi_i \tag{3.6}$$

where ϕ_n and ϕ_i can eventually be calculated using Henderson-Hasselbach equations. Intermedia transport rates (advection, partitioning) under non-equilibrium conditions and degradation rates are be described by a first-order law (Eq. 3.7):

$$dm/dt = Ta_t \tag{3.7}$$

where T (m^3 h^{-1}) is defined in analogy with the transport coefficient D for fugacity.

The first application of the activity approach was the regional model (MAMI–Multimedia Activity Model for Ionics; Franco and Trapp, 2010). Recently, activity has been used to replace fugacity as state variable in the multimedia WWTP model Activity SimpleTreat (Franco et al., 2011). This model was developed as a revisited version of SimpleTreat 3.1, maintaining its major

features (e.g., WWTP size and structure; Fig. 3.1) but aiming at improving fate predictions of ionizable XTCs. Activity SimpleTreat accordingly includes updated K_{oc} regressions for ionizable organic chemicals (Franco et al., 2009). In this PhD thesis (**Paper IV**), Activity SimpleTreat was applied for the first time for fate prediction of ionizable XTCs in WWTPs.

Overall, multimedia fate models based on either fugacity or activity concepts were developed as generic tools for exposure assessment under steady-state conditions. The use of multimedia models thus aims at predicting, in an ideal conventional WWTP, steady-state XTC elimination and concentrations (effluent, sludge) to further estimate exposure levels in environmental recipients (freshwater, soil). Predictions are meant to be obtained using limited input data, i.e. (i) steady-state XTC emissions to WWTPs from e.g., regional or catchment consumption data; and (ii) properties of the chemical investigated (molecular weight, vapour pressure or Henry's law constant, solubility, dissociation constants, octanol-water partition coefficient and biodegradation rate in activated sludge).

All these features made multimedia models suitable for use in decision support tools frameworks. Specifically, SimpleTreat 3.1 is currently part of the European Union System for Evaluation of Substances (EUSES; EC, 2004), used for environmental risk assessment of XTCs according to the REACH regulation. Notably, the predictive capacity of multimedia models has been often tested in terms of removal efficiency with measurements in pilot- and full-scale WWTPs (Table 3.1). On the contrary, few attempts of predicting XTC concentrations using real emission data exist (Artola-Garicano et al., 2003b; Cunningham et al., 2012). In **Paper IV**, we aimed at filling this knowledge gap by testing Activity SimpleTreat and predicting concentrations (in sewage sludge and effluent) using real emission data from different geographical regions of the EU.

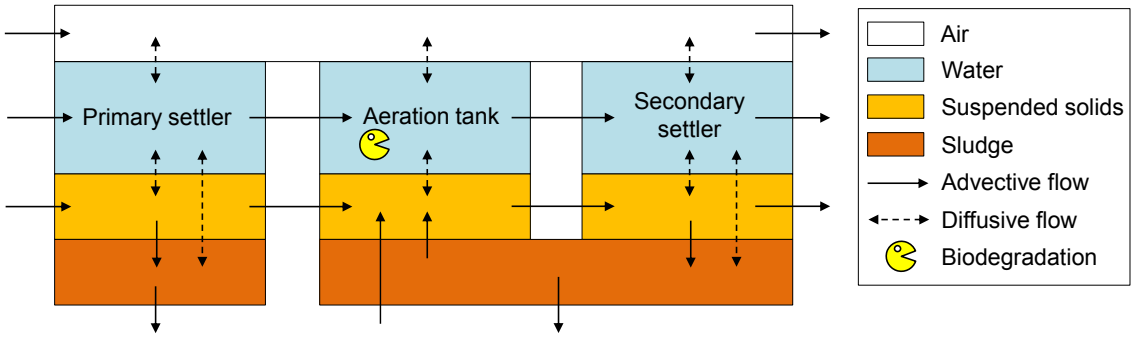


Figure 3.1. Layout of the ideal WWTP described in SimpleTreat and Activity SimpleTreat (adapted from EChA, 2008).

3.2 Concentration-based models

Concentration-based models refer to rather broad category of models, employing concentration as state variable to describe the fate of XTCs during wastewater treatment. Similarly to multimedia models, XTC concentrations are defined in aqueous, sorbed and (when relevant) gaseous phase. Models selected (Table 3.1) are characterized by varying levels of model complexity and data requirement, which will be considered for model description in this section. The heterogeneity of concentration-based models is further highlighted by the number of different notations used to describe the same state variable, e.g., aqueous concentration (S_{NTA}, S, C, C_W, $C_{W,bulk}$, C_f, C_{free}, S_{XOC}, $C_{dissolved}$, C_{LI}, S_{PhAC}, S_{MP}) and sorbed concentration (X_{NTA}, q, X, C_S, C_{OC}, X_{XOC}, C_{SL}, c_P, c_{DCM}, C^*, X_{MP}).

In steady-state concentration-based models, analytical solutions, derived from mass balance equations, are used to predict XTC concentrations and removal efficiencies. Although model application in laboratory-scale studies was tested (Birch, 1991; Suarez et al., 2010), steady-state models have been mainly associated to full-scale fate predictions. Steady-state generic WWTP models have been developed and applied for: (i) scenario simulations in an ideal WWTP (Byrns, 2001); (ii) model validation with measured data, following model implementation of actual design parameters from the WWTPs investigated (Namkung and Rittmann, 1987; WW-TREAT by Cowan et al., 1993). In both cases, limited data was required in terms of model input (chemical properties, retrieved from literature or estimated via targeted experiments) and of full-scale measurements (assuming steady-state WWTP operation). Steady-state full-scale concentration-based models thus exhibit significant similarities to multimedia WWTP models.

Dynamic concentration-based models are sets of ordinary differential equations, describing specific process rates (included in a Gujer-Petersen matrix) and overall mass balances in the systems studied. Dynamic fate models were often developed as extension of ASMs (see Table 3.1), thus providing a simultaneous description of conventional pollutant and XTC removal processes during wastewater treatment.

To gain process understanding under controlled conditions, dynamic models were used for fate predictions in laboratory-scale systems. Models were calibrated to data obtained in batch (e.g., Artola-Garicano et al., 2003a; Urase and Kikuta, 2005) or continuous flow experiments (e.g., Fernandez-Fontaina et al., 2014). Estimated parameter values were used to compare chemicals, operational conditions tested and to derive implications for removal in full-scale WWTPs (e.g., Joss et al., 2006a).

Dynamic models were also applied for fate predictions in selected pilot- and full-scale WWTPs, mostly focusing on secondary treatment. The application of dynamic models aimed at in-depth assessing XTC fate considering that: (i) operation in WWTPs is dynamic; and (ii) most importantly, influent loadings of XTCs originating from household and industrial releases exhibit significant short-term (Melcer et al., 1994; Joss et al., 2005; Ort and Gujer, 2006; Ort et al., 2010a; Plósz et al., 2010b) and long-term (e.g., seasonal; Coutu et al., 2013; Marx et al., 2015) variations. Dynamic models developed, e.g., by Melcer et al. (1994; TOXCHEM) and Lindblom et al. (2009) were calibrated to continuous pilot-scale monitoring data. Furthermore, comprehensive dynamic modelling exercises (e.g., ASM-X; Plósz et al., 2010a, 2012) combined (i) parameter estimation with results from targeted (batch) experiments; and (ii) model validation with full-scale monitoring data. Clearly, refined predictions obtained with dynamic models required more experimental data than for steady-state and multimedia models.

More recently, full-scale dynamic models have been employed in integrated dynamic modelling tools (Lindblom et al., 2006; Vezzaro et al., 2014; Snip et al., 2014), simulating XTC fate at catchment scale and describing fate and transport in sewer systems, WWTPs and recipients. Generic WWTP layouts were considered using, e.g., Benchmark Simulation Model No. 1 (e.g., BSM1; Alex et al., 2008). To date, application of integrated models in WWTP catchments has been limited to realistic scenario simulations.

Several dynamic models explicitly described the influence of redox conditions on the fate of XTCs in wastewater systems (Joss et al., 2004; Wick et

al., 2009; Xue et al., 2010; Plósz et al., 2010a, 2012), differently from multimedia models and most of steady-state models. Furthermore, elimination under aerobic nitrifying conditions was specifically assessed to explore the possibility of cometabolic XTC biotransformation by nitrifying bacteria (Wick et al., 2009; Plósz et al., 2012; Sathyamoorthy et al., 2013; Fernandez-Fontaina et al., 2014).

The Activated Sludge Modelling framework for Xenobiotics (ASM-X) is a relevant example of dynamic concentration-based model. ASM-X was developed as an extension of ASM1 (Henze et al., 1987) and successfully calibrated and used for fate prediction of pharmaceuticals (antibiotics: Plósz et al., 2010a; diclofenac and carbamazepine; Plósz et al., 2012) in the Bekkelaget full-scale WWTP (Oslo, Norway). ASM-X predictions were based on preliminary model identification and calibration using batch experimental data and validation with full-scale concentration data from continuous monitoring ASM-X was further implemented in integrated catchment models (Snip et al., 2014) and to specifically assess fate of illicit drugs in upstream sewer systems (Plósz et al., 2013a). State variables are represented by the concentrations of the aqueous parent XTC fraction (C_{LI}), of the sorbed parent fraction (C_{SL}) and of the retransformable fraction (C_{CJ}), identifying a range of chemicals forms capable of undergoing retransformation to C_{LI} (see 2.2.3). Fate processes are separately described for different redox conditions and included biological transformation of C_{LI}, retransformation of C_{CJ} back to C_{LI} fraction and sorption/desorption onto and from activated sludge. Process rates are described using pseudo-first-order (biotransformation, retransformation, sorption) or first-order rate equation (desorption) with respect to XTC concentrations. Relevant parameters thus include the biotransformation rate constant k_{bio} (L $gTSS^{-1}$ d^{-1}), the retransformation rate constant k_{dec} (L $gTSS^{-1}$ d^{-1}), the solid-liquid partition coefficient K_d (L $gTSS^{-1}$) and the desorption rate k_{des} (d^{-1}).

In this PhD thesis, we evaluated the extension of the ASM-X framework to describe sorption of ionizable XTCs (**Paper I**) and its application to characterize XTC biotransformation in laboratory-scale MBBRs (**Paper II**). Furthermore, full-scale ASM-X predictions were compared to literature removal efficiency data and used to assess the impact of retransformation in XTC elimination (**Paper III**).

3.3 Modelling fate processes: biotransformation

Among the multimedia and concentration-based models reviewed (Table 3.1), agreement can be found on the approaches used to simulate fate processes. Specifically, (i) solid-liquid partitioning is described using K_d-based linear model (Eq. 2.1); (ii) volatilization and stripping are not relevant mechanisms when considering pharmaceuticals and biocides (XTCs assessed in this PhD thesis); and (iii) retransformation processes should be considered when XTCs are present in conjugated form and/or parent XTCs can be formed from other XTCs (see, e.g., natural estrogens). On the contrary, several different approaches have been tested when modelling XTC biotransformation/biodegradation during wastewater treatment.

The first discrepancy is represented the phase, in which biotransformation is assumed to take place. Most of the models reviewed described biotransformation of XTCs rigorously in aqueous phase, considering the aqueous concentration as the only biologically available (Alexander, 2000; Schwarzenbach et al., 2003). The aqueous fraction can include both the freely dissolved fraction and the fraction sorbed onto dissolved and colloidal matter (Delgadillo-Mirquez et al., 2011). Alternative approaches have been explicitly addressed in Table 3.1 (see 'Notes') and include: (i) biotransformation of only the sorbed concentration (e.g., the 'two-phase model' by Urase and Kikuta, 2005); and (ii) biotransformation of both aqueous and sorbed concentrations (e.g., WW-TREAT by Cowan et al., 1993). In both cases, sorption is considered as an initial mechanism making XTCs available for biological transformation (bio-sorption approach). This hypothesis was considered to describe sorption and transformation in sludge EPS (Khunjar and Love, 2011), however major evidences for bacterial cells are lacking. Nevertheless, a few detailed literature studies considered the hypothesis as unlikely (e.g., Delgadillo-Mirquez et al., 2011). Notably, in SimpleTreat and Activity SimpleTreat modellers can select whether biotransformation takes place in aqueous phase or both in aqueous and sorbed phase.

The major difference among all models reviewed is certainly the mathematical description of the rate of biotransformation/biodegradation. In the following paragraphs, we will focus on models describing the biotransformation of only aqueous XTC concentration (C, μg L^{-1}). The term X will generically indicate the concentration of biomass in terms of TSS (gTSS L^{-1}; e.g., Joss et al., 2006a,b), VSS (gVSS L^{-1}; e.g., Fernandez-Fontaina et al., 2014) or active heterotrophic or autotrophic biomass (gCOD L^{-1}; e.g., Majewsky et al.,

2011a; Sathyamoorthy et al., 2013). A common assumption used in all models, describing biotransformation in suspended growth systems, is that uptake or diffusion into cells is not a rate limiting process, and XTC concentrations in cells equals the concentration in bulk aqueous phase (Schwarzenbach et al., 2003).

Monod-based kinetic expressions with respect to XTC concentration were considered to describe biotransformation as a growth process for a population of specialist degraders X_C (Eq. 3.8; Siegrist et al., 1989; Lindblom et al., 2006, 2009):

$$\frac{dC}{dt} = -\frac{\mu_{C,\max}}{Y_C}\frac{C}{(C+K_C)}X_C \tag{3.8}$$

where $\mu_{C,max}$, Y_C and K_C denote the maximum growth rate (d^{-1}), the yield coefficient ($gX_C\ gC^{-1}$) and the affinity constant ($mgC\ L^{-1}$) for growth on XTCs.

Acknowledging that XTCs are secondary susbtrates, present in concentrations significantly lower than their affinity constant, and that their biotransformation can be catalysed by non-specific enzymes widely present in the microbial population X (Alexander, 1985; Namkung and Rittmann, 1987; Nyholm et al., 1996; Alvarez-Cohen and Speitel, 2003; Schwarzenbach et al., 2003), several models (e.g., Joss et al., 2006a,b; Wick et al., 2009) included a pseudo-first-order rate equation (Eq. 3.9) for biotransformation:

$$\frac{dC}{dt} = -k_{bio}CX \tag{3.9}$$

where k_{bio} is defined as the biotransformation rate constant ($L\ gX^{-1}\ d^{-1}$). Eq. 3.9 represents a simplification of the generic Monod equation at low concentrations ($C<<K_C$). The attribute 'pseudo' refers to the dependency from the term X, assumed constant when considering negligible biomass growth during the investigation (Joss et al., 2006a). When used as extension of models (e.g., ASMs) describing growth processes, Eq. 3.9 can be defined as a second-order rate equation, effectively describing cometabolic XTC transformation (Liu et al., 2014). In order to account for the instantaneous adjustment of partitioning equilibria via desorption during biotransformation, Eq. 3.9 can be rewritten (Joss et al., 2006a):

$$\frac{dC}{dt} = -\frac{k_{bio}}{(1+K_d X)}CX \tag{3.10}$$

We note that Eq. 3.10 is a compact version of a partitioning-biotransformation model, employing Eq. 3.9 and separate process equations for sorption and desorption.

Using the assumption of negligible biomass growth (constant X) during batch experiments or continuous operation, a first-order biotransformation rate was also used (Eq. 3.11; Struijs et al., 1991; Artola-Garicano et al., 2003a; Vezzaro et al., 2014):

$$\frac{dC}{dt} = -k'_{bio} C \tag{3.11}$$

where k'_{bio} indicates the biotransformation rate (d^{-1}).

Based on experimental observations, a number of models explicitly considered the beneficial effect of growth substrate availability on XTC biotransformation rates (Delgadillo-Mirquez et al., 2011; Plósz et al., 2012; Sathyamoorthy et al., 2013; Fernandez-Fontaina et al., 2014). This approach—also known as reductant model (Alvarez-Cohen and Speitel, 2001; Liu et al., 2014)—was originally inspired by the model developed by Criddle (1993). Biotransformation was accordingly described (Eq. 3.12) as two-rate process in the presence (enhanced rate) and in the absence of growth substrate S:

$$\frac{dC}{dt} = -\left(q\frac{S}{S+K_S} + k \right)\frac{C}{C+K_C} X \tag{3.12}$$

where q and k are the maximum XTC utilization rate constants (gC gX^{-1} d^{-1}) in the presence and in the absence of growth substrate, respectively. The parameter q lumps maximum growth rate (μ_{max}, d^{-1}), yield (Y, gX gS^{-1}) over the growth substrate and the stoichiometric transformation yield (T, gC $gS^{-1;}$ Alvarez-Cohen and Speitel, 2001) (Eq. 3.13):

$$q = T\frac{\mu_{\max}}{Y} \tag{3.13}$$

The concept of rate enhancement was justified by the regeneration of reductants (e.g., NADPH) used by oxidative enzymes via the degradation of growth substrates, thus providing a benefit for XTC biotransformation (Alvarez-Cohen and Speitel, 2001). Simplified versions of Eq. 3.12 have been implemented in the mentioned XTC fate models. Assuming pseudo-first-order kinetics with respect to XTC concentration ($C<<K_C$), the reductant model (Eq.

3.14) was used to describe biotransformation in mixed culture (Plósz et al., 2012) and nitrifying culture systems (Sathyamoorthy et al., 2013):

$$\frac{dC}{dt} = -\left(q_C \frac{S}{S + K_S} + k_{bio} \right) CX \tag{3.14}$$

where q_C and k_{bio}, defined as biotransformation rate constant (L gX^{-1} d^{-1}) in the presence and in the absence of growth substrate, are equivalent to q and k normalized by K_C. Alternatively, under the assumption of non-limiting growth substrate availability, Fernandez-Fontaina et al. (2014) described biotransformation as a one-rate cometabolic process (Eq. 3.15):

$$\frac{dC}{dt} = -\left(q \frac{S}{S + K_S} \right) \frac{C}{C + K_C} X \tag{3.15}$$

Growth substrates can also compete with XTCs for the same nonspecific enzymatic sites, thus hindering XTC biotransformation rates (Alvarez-Cohen and Speitel, 2001). XTC biotransformation rate with competitive inhibition by growth substrate was accordingly described in Eq. 3.16 (Criddle, 1993; Orozco, 2008):

$$\frac{dC}{dt} = -k \frac{C}{C + K_C \left(\frac{S + K_I}{K_I} \right)} X \tag{3.16}$$

where K_I (mgS L^{-1}) is defined as inhibition constant. Plósz et al. (2010a) used a simplified version of Eq. 3.16 to describe competitive inhibition effects on XTC biotransformation (Eq. 3.17):

$$\frac{dC}{dt} = -k_{bio} CX \left(\frac{K_I}{S + K_I} \right) \tag{3.17}$$

A non-competitive inhibition model was instead used to describe mixed substrate utilization by specialist degraders (Eq. 3.18; Siegrist et al., 1989)

$$\frac{dC}{dt} = -\frac{\mu_{C,\max}}{Y_C} \frac{C}{C + K_C} X \left(\frac{K_I}{S + K_I} \right) \tag{3.18}$$

Conversely, XTC inhibition of growth substrate utilization (competitive: Sathyamoorthy et al., 2013; non-competitive: Vasiliadou et al., 2013) has been also described—a less probable situation considering the significant concentration differences at environmental conditions (Liu et al., 2014).

Although limited experimental evidences exist, the temperature dependence of biotransformation rates or rate constants was implemented in a number of models (Joss et al., 2006b; Franco et al., 2011, 2013b) using the Arrhenius equation (Eq. 3.19):

$$k_T = k_{T_{ref}} \theta^{(T - T_{ref})} \tag{3.19}$$

where k denotes a generic biotransformation rate parameter (first-order rate, pseudo-first-order rate constant), T_{ref} a reference temperature (typically 20°C), T the actual temperature (°C), at which biotransformation was assessed, and θ the Arrhenius coefficient (1.03–1.09: Joss et al., 2006b; 1.072: Franco et al., 2013b). Based on experimental evidences (e.g., Castiglioni et al., 2006), the impact of temperature on the biotransformation of furosemide in activated sludge was considered in **Paper IV**.

Dependency of biotransformation rate constants from pH was further described (Gulde et al., 2014) to account for cell uptake and thus biotransformation of only the neutral fraction of XTCs (Eq. 3.20):

$$k_{pH} = \phi_n k_{pH,ref} \tag{3.20}$$

where ϕ_n denotes the pH-dependent fraction of neutral XTC (Eq. 2.4) and $k_{pH,ref}$ the maximum theoretical rate constant when $\phi_n = 1$.

Table 3.1. Summary of models describing the fate of XTCs in (biological) wastewater treatment systems.

	Popular name	State	SS	D	Processes: Biotransf Biodeg	Sorp/Des	Retransf	Volatil/ Stripp	Ioniz	Other	Scale of application / System boundaries: Lab	Pilot	Full	Redox cond	XTCs	Parameter source	Notes
1. Multimedia models																	
Strujis et al. (1991)	Simple Treat	Fugacity			First-order T correction	Linear			Optional					Aer	Halogenated hydrocarbons Surfactants Pharmaceu-ticals Fragrances	Literature	Sorp/Des and Volatil only for neutral species
Strujis (1996)	v. 3.0																
EC (2004)	v. 3.1				First-order or Monod-based Specialist degraders T correction												K_{ow}-based regressions for K_{oc} (Sablijc et al., 1995)
Franco et al. (2013a,b)	v. 3.2															Continuous-flow experiments	Revised regressions for sorption of ionized species
Struijs (2014)	v. 4.0															Literature	
Artola-Garicano et al. (2003b)	Simple Treat 3.0	Fugacity			First-order	Linear								Aer	Fragrances	Batch experiments	
Clark et al. (1995)	STP	Fugacity			First-order	Linear								Aer	Aromatics PAHs Halogenated hydrocarbon Phenols Pesticides	Literature	Phases in each tank (air, water, solids) in equilibrium. Biotransf in solid phase
Khan and Ongerth (2004)	STP	Fugacity			First-order	Linear	(In-sewer complete deconjuga-tion)	Neglig.					+ Excre-tion and sewer	Aer	Pharmaceu-ticals	Literature, software estimation	Sorp/Des only for neutral species
Seth et al. (2008)	STP-EX (Update of STP and Simple Treat 3.0)	Fugacity			First-order	Linear								Aer	VOCs Phenols PAHs Phthalates Pesticides Halogenated hydrocarbons	Literature and expert estimation	Biotransf in aqueous, sorbed or both phases. Sorp/Des and Volatil. only for neutral species
Franco et al. (2011)	Activity Simple Treat	Activity			First-order or Mondo-based Specialist degraders	Linear								Aer			Sorp of neutral and ionic species (Franco and Trapp, 2008; Franco et al., 2009)
Cunningham et al. (2012)	PhATE	Fugacity			First-order	Linear								Aer	Estrogens Pharmaceuti-cals	Literature	Extension of PhATE with SimpleTreat 3.1

	Popular name	State	SS	D	Processes: Biotransf Biodeg	Sorp/Des	Retransf	Volatil/ Stripp	Ioniz	Other	Scale of application / System boundaries: Lab	Pilot	Full	Redox cond.	XTCs	Parameter source	Notes
2. Concentration-based models																	
Namkung and Rittmann (1987)		Conc (S, q)			Pseudo-first-order	Linear								Aer	Aromatics, Halogenated hydrocarbons	Literature	Scenario simulations Validation with WWTP data
Siegrist et al. (1989)		Conc (S_{NTA}, X_{NTA}, $X_{B,NTA}$)			Specialist degraders Monod-based Primary substrate inhibition	Linear								Aer (N)	Detergent builders	Literature Batch experiments (sorption)	ASM1 extension
Birch (1991)		Conc (S)			Specialist degraders Monod-based									Aer (N)	Surfactants Detergent builders	Continuous-flow experiments	XTCs growth substrates
Cowan et al. (1993)	WW-TREAT	Conc (C, C_s, C_t)			First-order	Linear		Neglig.						Aer	Surfactants Detergent builders	Literature Batch experiments	Biotransf of aqueous and sorbed conc
Melcer et al. (1994), Monteith et al. (1995)	TOX CHEM	Conc (C, q)			Pseudo-first-order	Linear								Aer	Halogenated hydrocarbons Aromatics Pesticides	Pilot-scale experiments	
Byrns (2001)		Conc (C_w)			Pseudo-first-order	Linear								Aer	Aromatics, PAH, Halogenated hydrocarbons Insecticides	Only simulation study	Scenario simulations in ideal WWTP
Artola-Garicano et al. (2003a)		Conc (C_{free}, C_{oc}, C_{total})			First-order	Linear								Aer	Fragrances	Batch experiments	Model calibration to measured aqueous and total conc
Joss et al. (2004)		Conc ($C_{W,bulk}$, C_S)			Pseudo-first-order	Linear	E2→E1, E1→E2 Deconju-gation Pseudo-first-order							Aer (N) Anox Anaer	Estrogens	Batch experiments	
Urase and Kikuta (2005)	Two-phase model	Conc (C_W, C_S)			Pseudo-first-order	Linear	Pseudo-first-order (E2→E1)							Aer	Estrogens Endocrine disrupters Pharmaceu-ticals Fragrances	Batch experiments	Biotransf of sorbed conc. Model application to EBPR
Xue et al. (2010)														Aer (N) Anox Anaer		Batch/full-scale experiments	

Joss et al. (2006a,b)		Conc (S, X, C_{air})		Pseudo-first-order	Linear								Aer (N)	Pharmaceuti-cals X-ray contrast media	Batch experiments	WWTP removal scenarios. Application by Maurer et al. (2007), Abegglen et al. (2009)
Lindblom et al. (2006)		Conc (S_{XOC}, X_{XOC}, $X_{B,XOC}$)		Specialist degraders, Monod-based	Linear							+ sewer	Aer	Endocrine disrupters PAH	Only modelling study	ASM1 + WATS extension Growth on XTCs, primary substrate
Lindblom et al. (2009)		Conc (S_{XOC}, X_{XOC}, $X_{B,XOC}$)		Specialist degraders, Monod-based	Linear								Aer (N)	Endocrine disrupters	Continuous-flow experiments	ASM1 extension. Growth on XTCs only
Wick et al. (2009)		Conc (C_w)		Pseudo-first-order T correction	Neglig.								Aer (N) Anox	Pharmaceuti-cals	Batch experiments	Anox removal with scenario simulation
Suarez et al. (2010)		Conc ($C_{dissolved}$ C_{tot}, C_{air})		Pseudo-first-order	Linear								Aer (N) Anox	Estrogens, Pharmaceuti-cals Fragrances	Continuous-flow experiments	Estimation of elimination pathways
Plósz et al. (2010, 2012)	ASM-X	Conc (C_{LI}, C_{SL}, C_{CJ}, C_{SI})		Pseudo-first-order Primary substrate enhanc. or inhibition	Linear	Pseudo-first-order		Seque-stration in solids					Aer (N), Anox	Pharmaceuti-cals	Batch experiments, full-scale sampling	ASM1 extension. Application in integrated full-scale systems by Snip et al. (2014).
Delgadillo-Mirquez et al. (2011)	Four compart-ment model	Conc (C_f, C_g, c_{DCM}, c_P)		Monod-based Primary substrate enhanc.	Linear Sorption to particles and DCM								Anaer	PAH	Continuous-flow experiments	Application for anaer digestion Biotransf of aqueous conc (dissolved + sorbed on DCM)
Ottmar et al. (2012)		Conc (C, S)		Zero-order or pseudo-first-order Primary substrate enhanc.	Linear								Aer	Pharmaceuti-cals	Continuous-flow and batch experiments	Extension of ASM model for COD removal only
Sathyamoorthy et al. (2013)		Conc (S_{PhAC})		Pseudo-first-order Primary substrate enhanc.	Linear								Aer (N)	Pharmaceuti-cals	Batch experiments	Extension of ASM model for two-step nitrification. XTC inhibition of NH_4 oxidation
Vasiliadou et al. (2013) Vasiliadou et al. (2014)		Conc (C, C^*)		Pseudo-first-order	Linear			Diffusion in biofilm					Aer (N)	Pharmaceuti-cals	Batch and sequencing-batch experiments	Extension of process model for simultaneous growth (COD, N) XTC inhibition of biomass growth

Vezzaro et al. (2014) (see also De Keyser et al., 2010; Cloutier et al., 2012)	IUWS_ MP	Conc (S_{MP}, X_{MP})			First-order	Linear				Hydrolysis Settling Filtration Photolysis			+ Sewer Sludge treatm. River fate	Aer (N) Anox Anaer	Plasticizers Endocrine disrupters Herbicides PAH Estrogens		Extension of ASM2d and fate models (sewers, rivers, sludge, stormwater) Scenario simulations with ideal (bench-mark) WWTP
Fernandez-Fontaina et al. (2014)		Conc (C_W, C_S)			Monod-based, Primary substrate enhanc.	Linear								Aer (N)	Pharmaceuti-cals Fragrances	Continuous-flow experiments	Extension of ASM1-based nitrification model (measured nitrification rate)
Gulde et al. (2014)		Conc (C_W, C_t)			Pseudo-first-order	Linear				Abiotic transf (First-order)				Aer	Pharmaceuti-cals Pesticides Fungicides	Batch experiments	Ionization patterns used to re-interpret estimated k_{Bio} at different pH
Pomiés et al. (2015)		Conc (S_{mp}, X_{mp})			Pseudo-first-order Primary substrate enhanc. or inhibition	Linear								Aer (N) Anox	Pharmaceuti-cals	Batch experiments	Biotransf of aqueous and sorbed conc. Inhibition/enhanc. switch functions depending on conditions tested

Abbreviations: Conc = concentration; SS = steady-state; D = dynamic; Biotransf = biotransformation; Biodeg = biodegradation; Sorp/Des = sorption/desorption; Retransf = retransformation; Volatil/Stripp = volatilization/stripping; Ioniz = ionization; Redox cond = Redox conditions; T correction = temperature correction; Aer = aerobic; Aer (N) = aerobic (nitrifiying); Anox = anoxic; Anaer = anaerobic; enhanc. = enhancement; Neglig. = negligible; PAH = polycyclic aromatic hydrocarbons; E1 = estrone; E2 = 17β-estradiol; ASM = Activated Sludge Model; DCM = dissolved and colloidal matter.

Additional notes: relevant process rates or expressions for biotransformation/biodegradation and sorption are included in the respective box for each model. For biotransformation/biodegradation, the notations 'First-order' (Eq. 2.14), 'Pseudo-first-order' (Eq. 2.12–2.13) and 'Monod-based' (Eq. 2.11) refer to the expressions used with respect to XTCs regardless of other terms in the rate equations used. The additional notations 'Primary substrate enhancement' and 'Primary substrate inhibition' refer to the inclusion of additional terms in the rate equation according to Eq. 2.15–2.18 and Eq. 2.19–2.21, respectively.

3.4 Beyond wastewater treatment: fate modelling in agricultural systems

As outlined in Chapter 1, XTCs can be released to agricultural systems via fertilization with manure or WWTP biosolids and/or irrigation with freshwater or reclaimed wastewater. In soil, XTC fate is essentially characterized by processes similar to the ones occurring during wastewater treatment: sorption and sequestration to solid matrices (soil organic matter and minerals), microbial and abiotic degradation (e.g., photolysis), and volatilization (when relevant). In addition, XTCs undergo advective transport with soil pore water, which can result in downward leaching to groundwater or uptake into plants via transpiration. Distribution into different plant compartments results from the transport of water and nutrients through the plant and mainly depends on XTC properties (e.g., ionization pattern, partitioning capacity) (Trapp, 2009). Although experimental evidences are still scarce, XTCs are likely metabolized and thus degraded in plants (Komossa et al., 1995).

In this PhD thesis, a dynamic soil-plant model was applied to predict the fate of ionizable XTCs and their uptake in winter wheat (**Paper IV**). The model includes previously listed fate processes by combining a *tipping buckets water balance model* (Trapp and Matthies, 1998) and a *dynamic plant uptake model* (Legind et al., 2011, 2012; Rein et al., 2011; Trapp and Eggen, 2013). A schematic presentation of the simulated system is given in Fig. 3.2, considering winter wheat as the investigated crop.

The thickness of an ideal soil volume (1 m) is discretized into five layers which are simulated as tipping buckets. Each layer is characterized by an upper and a lower limit for water storage, described by the field capacity and the permanent wilting point, respectively. Downward transport of dissolved XTCs follows downward water movement, which is determined by the water content of each layer. If the water content soil layer reaches field capacity, transport of water and dissolved XTCs occurs to the layer underneath. Concurrently, upward transport of water and dissolved XTCs occurs via transpiration to the plant (and, if relevant, subsequent evaporation). The dissolved XTC fraction is calculated based on the solid-liquid partition coefficient K_d in soil. The overall mass balance of the dissolved XTC fraction in soil includes: (i) input via sludge application or irrigation to topsoil (layer 1); (ii) leaching from upper layers (layers 2-5) and losses via leaching to lower layers or to groundwater (layers 1–5); (iii) transpiration to plant roots from each layer; (iv) sorption and biological degradation (first-order temperature-dependent

kinetics) in each layer. Transpiration to plant is simulated as a function of plant growth.

A cascade approach is used to solve analytically differential equations for the distribution of uptaken XTC into different compartments of the wheat plant (roots, stem, leaves and grains). Intra-compartment transport of dissolved XTCs is simulated to occur via the two tissues transporting water and nutrients in the plant, i.e. xylem (soil → roots → stem → leaves/grains) and phloem (leaves → stem → grains). Water flux with the xylem is typically one to two orders of magnitude larger than with the phloem (Trapp and Matthies, 1998; Rein et al., 2011). The mass balance for XTCs in each plant compartment includes: (i) inward and outward transport (when relevant) with xylem and/or phloem; (ii) dilution effect due to plant growth; and (iii) partitioning between plant compartments and xylem/phloem. XTC partitioning eventually determines the extent of the distribution and is described using a *cell model* (Trapp, 2004). According to the cell model, plant compartments are subdivided into water, lipids and proteins, i.e. major components of plant cells (cytosol and vacuoles). Overall, XTC partitioning between xylem/phloem and plant cells accounts for: (i) different pH conditions occurring in xylem, phloem and cell compartments; (ii) different sorption capacity exhibited by neutral and ionized XTC species; and (iii) the different affinities to cell lipids and proteins. With respect to (iii), equations have been defined to empirically estimate partition coefficients (L L^{-1}) to lipids of neutral ($K_{n,lipid}$, Eq. 3.21) and ionized species ($K_{i,lipid}$, Eq. 3.22) (Trapp et al., 1994):

$$K_{n,lipid} = 1.22 \cdot L \cdot K_{ow,n}^{b} \tag{3.21}$$

$$K_{i,lipid} = 1.22 \cdot L \cdot K_{ow,i}^{b} \tag{3.22}$$

and the partition coefficient to proteins ($K_{protein}$, Eq. 3.23):

$$K_{protein} = P \cdot K_{a,HSA} \tag{3.23}$$

where L (kg kg^{-1}) and P (mol L^{-1}) denote the lipid and protein content in each compartment, 1.22 is the density ratio between octanol and water, b accounts for the difference between octanol and plant lipids (0.77 for roots, 0.95 for stem, leaves and grains), $K_{ow,n}$ and $K_{ow,i}$ denote the octanol-water partition coefficient for neutral and ionized species, respectively, and $K_{a,HSA}$ (L mol^{-1}) defines the partition coefficient to human-serum albumin. The significance of adsorption to proteins was shown for a number of ionizable XTCs, being influential for their accumulation in plants (Macherius et al., 2012).

The dynamic soil-plant model was developed as a generic tool, requiring limited input (chemical properties, degradation rates) to predict plant uptake of XTCs in ideal scenarios. Nevertheless, the model found application in real scenarios through parameterization with soil and plant characteristics and weather data (Legind et al., 2012; Trapp and Eggen, 2013; Prosser et al., 2014b; Trapp, 2015). Besides winter wheat, dynamic uptake models have been developed and tested for other plants, e.g., root vegetables (Trapp, 2009, 2015) and fruit trees (Trapp, 2007).

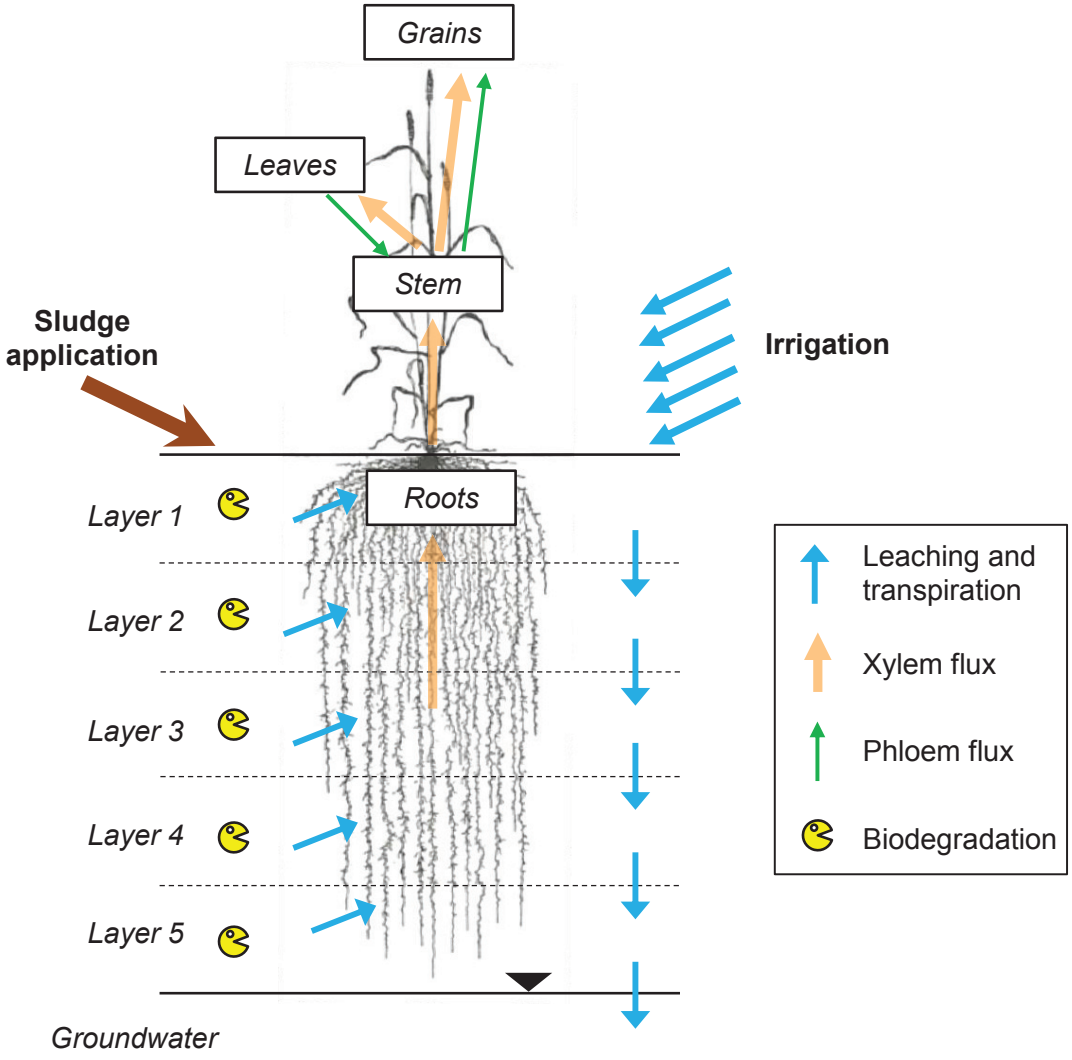


Figure 3.2. Schematic representation of the dynamic soil-plant uptake model, combining the tipping buckets water balance model and the dynamic plant uptake model. Solid arrows represent the main input and transport pathways for XTCs in the soil-plant system.

4 Sorption of zwitterionic ciprofloxacin onto activated sludge

Solid-liquid partitioning of ionizable XTCs onto solid matrices involves multiple mechanisms (2.2.2). Environmental conditions (pH, ionic strength) change the speciation of both sorbent and sorbate and can significantly influence partitioning of ionizable XTCs. Several studies focused on the importance of these factors on the sorption of ionizable XTCs in soils, whereas limited evidence has been obtained for wastewater systems. In particular, sorption of zwitterionic substances has been rarely assessed. Antibiotics from several classes (fluoroquinolones, tetracyclines) are in fact zwitterionic and can exhibit anionic and cationic charges when ionized. In **Paper I,** we investigated the influence of pH and iron salt dosing on the partitioning onto activated sludge. Iron salt ($FeCl_3$, $FeSO_4$) is typically added during secondary treatment for chemical phosphorus removal. The fluoroquinolone antibiotic ciprofloxacin was selected as example of ionizable XTC.

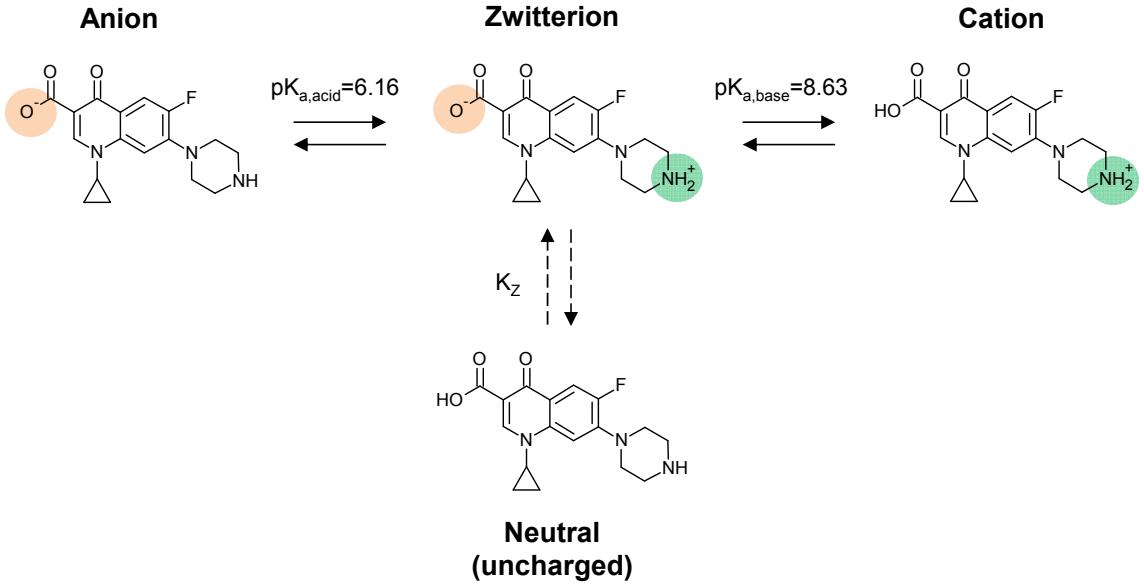


Figure 4.1. Ionized species of ciprofloxacin (pK_a values: Kümmerer, 2009a). Although zwitterionic chemicals can be typically present both as uncharged molecules and zwitterions (overall defining the neutral configuration), the former configuration is typically neglected for ciprofloxacin.

4.1 Properties of ciprofloxacin

Ciprofloxacin is a widely used fluoroquinolone antibiotic. As a zwitterionic chemical, ciprofloxacin can speciate as anion (acid pK_a=6.16), cation (base pK_a=8.63) zwitterion or neutral chemical (Fig. 4.1). Under relevant pH conditions, the neutral fraction is typically considered negligible as compared to the zwitterionic fraction (e.g., Kümmerer, 2009a), assuming K_Z=∞ (Eq. 2.10). The neutral fraction is here considered equivalent to the zwitterionic fraction ($\phi_n = \phi_{\pm}$, Eq. 2.6)

Based on reported pK_a values, the three ionized species (anion, cation and zwitterion) thus can be simultaneously present at pH conditions during secondary wastewater treatment (6.2–8.1: Helbling et al., 2012). Sorption of ciprofloxacin was found significant both in soils (Tolls, 2001; Thiele-Brun, 2003) and activated sludge (Golet et al., 2003; Lindberg et al., 2006; Plósz et al., 2010a) despite its low hydrophobicity (log$D$=-3.01 at pH= 7.4; ACD/logD, ilab.acdlabs.com). Thus, other mechanisms than hydrophobic partitioning are likely involved in the sorption of ciprofloxacin (Tolls, 2001; Vasudevan et al., 2009).

4.2 Experimental methods and results

Sorption experiments were performed to determine sorption of ciprofloxacin onto activated sludge: (i) at different pH (3.0, 6.3, 7.4, 8.0 and 12.0) under aerobic conditions; and (ii) with and without iron salt ($FeCl_3$, $FeSO_4$) addition at different redox conditions (aerobic, anoxic and anaerobic) and pH=6.3. In each experiment, activated sludge (30 mL; 3.0 gTSS L^{-1}) was spiked with ciprofloxacin at different initial concentrations (100–2400 µg L^{-1}). Following incubation for 23 h at 21°C, the residual aqueous concentration of ciprofloxacin was measured in filtered sludge supernatant (0.45 µm). The sorbed concentration of ciprofloxacin was determined as the difference between initial and final aqueous concentration, and sorption isotherms at different conditions were accordingly derived.

Experimental results are summarized in Figure 4.2 (a: pH influence; c: iron salt dosing influence). Freundlich equation (Eq. 2.2, n=0.67–1.33) was in all cases required to describe the sorption isotherms obtained (Fig. 4.2a,c). At typical wastewater pH (6.3–8.0), sorption was highly non-linear (n=0.67–0.77), as also shown for other ionizable XTCs (Hyland et al., 2012). To facilitate a comparison between the several cases assessed, solid-liquid parti-

tion coefficients K_d were estimated based on Freundlich equation at a theoretical aqueous concentration of 10 µg L^{-1} (Fig. 4.2b,d).

Sorption of ciprofloxacin onto activated sludge was significantly influenced by pH (Fig. 4.2a–b). Highest sorption (K_d=12.3 L $gTSS^{-1}$) was found at the iso-electric point (pH=7.4), when ciprofloxacin was mostly present as zwitterion (Fig. 4.2b). Similar sorption capacity was shown at pH=6.3 and 8.0 (K_d=3.7 L $gTSS^{-1}$). Sorption at pH=3 (K_d=2.4 L $gTSS^{-1}$) was considerably higher than at pH=12 (K_d=0.02 L $gTSS^{-1}$), indicating the least sorption for the anionic species. Negligible sorption by anions was found for several XTCs (Urase and Kikuta, 2005; Franco and Trapp, 2008; Tülp et al., 2009). Conversely, the highest sorption capacity was exhibited by the zwitterion (Fig. 4.2b).

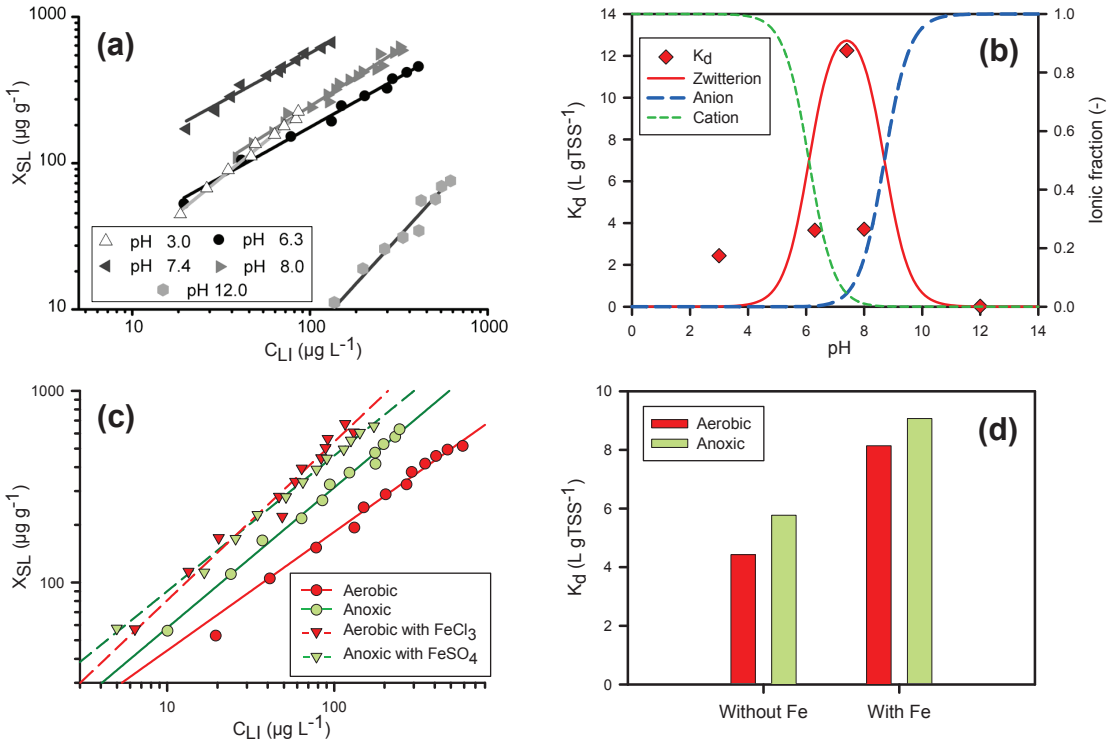


Figure 4.2. Sorption isotherms obtained at different pH (a) and different redox conditions with and without Fe salt addition (c). K_d values, estimated at the theoretical concentration C_{LI} = 10 µg L^{-1}, were compared to assess the effect of pH (b) and of Fe salt dosing at pH=6.3 (d).

In the absence of iron salt addition, sorption under anoxic conditions (K_d=5.8 L $gTSS^{-1}$) was higher than estimated under aerobic conditions (K_d=4.4 L $gTSS^{-1}$). Under both aerobic and anoxic conditions (Fig. 4.2c), the addition of Fe(II)- or Fe(III)-salts resulted in a consistent sorption increase (K_d=8.1 and

9.1 L $gTSS^{-1}$, respectively), although lower when compared to the effect of pH. On the contrary, no sorption increase due to iron salt addition was shown under anaerobic conditions (see **Paper I**). Enhancement of sorption was observed for other XTCs upon Fe and Al salt addition (Carballa et al., 2005).

Overall, these findings suggested that ciprofloxacin sorbed onto activated sludge via hydrophobicity-independent mechanisms. Based on previous evidences (Nowara et al., 1997; Gu and Karthikeyan, 2005; MacKay and Canterbury, 2005; Carrasquillo et al., 2008; Vasudevan et al., 2009; Pan et al., 2012; MacKay and Vasudevan, 2012) and on our experimental observations, we proposed the following mechanisms to describe sorption of ciprofloxacin at different pH and iron salt dosing conditions (Fig. 4.3).

Activated sludge surfaces typically carry negative charges due to the presence of anionic organic functional groups (Wang et al., 2000). Sludge surfaces thus exhibit high affinity for divalent cations (Ca^{2+} and Mg^{2+}) (Sobeck and Higgins, 2002). At pH=6–9, ciprofloxacin is present in the three ionized species. Cationic ciprofloxacin can sorb via Coulombic attraction onto negatively charged surfaces and cation exchange of the protonated amines. These mechanisms can also cause sorption of zwitterions, due the favourable charge distribution within the molecules—areas of opposite charge are located at opposite ends (Carrasquillo et al., 2008). In addition, zwitterions (and anions) can sorb through cation bridging via divalent cations with the deprotonated carboxyl. Upon dosing, iron cations can bind to negatively charged sludge surfaces. Zwitterions (and anions) can form stable ternary complexes with bound Fe^{3+} and, partially, Fe^{2+}, thus resulting in the observed increase of ciprofloxacin sorption. The extent of such increase depends on redox conditions, determining the oxidation state of Fe. At pH<6, sorption of cationic ciprofloxacin (the only species present) is likely limited by the reduction of negative charges onto sludge surfaces (pK_a=6.29; Wang et al., 2000). At pH>9, ciprofloxacin is speciated as anion, and Coulombic repulsion with negatively charged sludge surface significantly reduces the sorption capacity. We note that Coulombic repulsion of anionic ciprofloxacin may also predominate over sorption via cation bridging and complexation at pH=6–9.

It can be thus concluded that solid-liquid partitioning of ciprofloxacin is mainly an adsorption process (Schwarzenbach et al., 2003) determined to the greatest extent by the presence of the antibiotic as zwitterion. Zwitterionic ciprofloxacin can undergo sorption via multiple mechanisms, justifying our experimental observations.

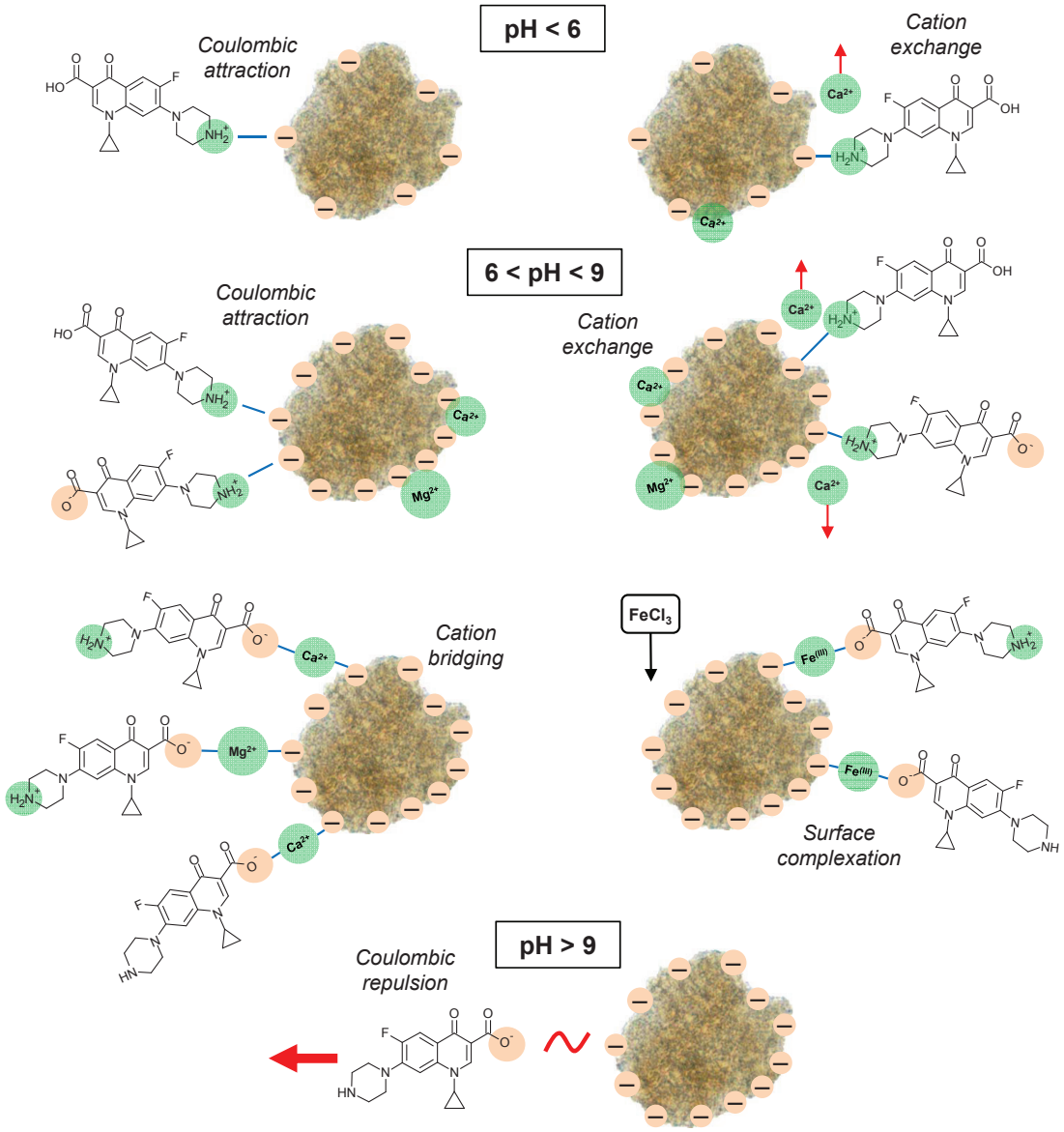


Figure 4.3. Mechanisms proposed to describe sorption of ciprofloxacin at different pH and iron salt dosing conditions onto activated sludge (activated sludge floc: adapted from Comeau, 2008).

4.3 Modelling implications

Based on experimental results, extensions of the ASM-X framework are proposed to describe sorption of ionizable XTCs. Experimental results showed the non-linearity of ciprofloxacin sorption under a range of conditions. Freundlich equation was required to describe sorption of other ionizable XTCs onto activated sludge (Hörsing et al., 2011; Hyland et al., 2012).

Freundlich-based sorption rate equation (Eq. 4.1) thus can be considered, e.g., as extension of ASM-X model:

$$dC_{LI}/dt = -k_{des} K_f C_{LI}^n X_{SS} \quad (4.1)$$

where k_{des} denotes the desorption rate (d^{-1}). Eq. 4.1 can provide a generalized description of XTC sorption at low and high concentrations, which could be beneficial when considering short-term dynamics (e.g., in influent concentration). However, Eq. 4.1 increases the structural complexity of the model and its application may be challenging when pH significantly impacts sorption.

An effective way of describing the influence of pH on partitioning is defined by separately accounting for linear sorption of different ionic species. For ciprofloxacin, the resulting K_d at different pH conditions would be accordingly described using (Eq. 4.2):

$$K_d(pH) = \phi_+ K_{d,+} + \phi_\pm K_{d,\pm} + \phi_- K_{d,-} \quad (4.2)$$

where ϕ_+, $\phi_\pm$, ϕ_- identify the ionic fractions and $K_{d,+}$, $K_{d,\pm}$, $K_{d,-}$ the partition coefficients of the cationic, zwitterionic and anionic species, respectively. No limitation of negative surface charge is considered, a reasonable assumption for pH conditions in activated sludge systems. Parameterization of Eq. 4.2 (i.e., experimental determination of $K_{d,+}$, $K_{d,\pm}$, $K_{d,-}$) may be required at different redox conditions and upon iron salt dosing. Eq. 4.2 can be applied to describe sorption of other ionizable XTCs having similar characteristics to ciprofloxacin (e.g., fluoroquinolones), with the possibility of neglecting K_{d-}. The partitioning submodel defined in Eq. 4.2 was further applied in **Paper IV** to predict the removal of ciprofloxacin in a generic WWTP.

Overall, both model extensions are meant to provide a refined description of the solid-liquid partitioning of ciprofloxacin (and other zwitterionic XTCs) onto activated sludge, supporting observations of this and future experimental studies.

5 Biotransformation of XTCs in single- and three-stage pre-denitrifying MBBRs

Conventional systems for biological wastewater treatment have been generally proved not efficient in terms of XTC elimination. A number of innovative technologies and reactor design solutions have thus been investigated for the improvement of XTC removal. Among these solutions, staging of biological reactors has been hypothesized to optimize pollutant removal processes based on reaction kinetics principles (Scuras et al., 2001; Joss et al., 2006a; Plósz, 2007; Grady et al., 2011). In staged 'sub-reactors', biomass is further exposed to significantly different substrate loading, availability and composition, as reactor staging induces a declining gradient in organic substrate loading and heterogeneity in the influent to subsequent sub-reactors. Such conditions may eventually lead to biomass adaptation or specialization, with improved utilization kinetics as compared to single-stage reactors (Plósz and Vogelsang, 2012). In addition, enhanced biotransformation kinetics were found for a number of XTCs in moving bed biofilm reactors (MBBRs), possibly resulting from prolonged biomass retention and enrichment of slowly growing bacteria (Falås et al., 2012, 2013).

In **Paper II**, we investigated the elimination of pharmaceuticals in two laboratory-scale MBBR configurations, i.e. single-stage (U) and three-stage (S1 + S2 + S3) MBBRs (Fig. 5.1). The two systems were designed for denitrification of pre-clarified municipal wastewater without addition of external COD and without spiking of XTCs. The three-stage MBBR configuration, designed according to Plósz (2007), could potentially combine the benefits of MBBRs and reactor staging in terms of XTC removal. During continuous-flow operation, the two MBBR systems were: (i) run in parallel under the same continuous-flow feeding conditions (pre-clarified municipal wastewater from Mølleåværket WWTP, Lundtofte, Denmark with external nitrate dosing); (ii) designed to have the same total operating volume (6 L), hydraulic residence time (8.9 h) and filling ratio (33%; K1 carriers, AnoxKaldnes, Lund, Sweden). Excess nitrate (KNO_3, influent concentration = 103 mgN L^{-1}) was continuously dosed to both MBBR systems in order to ensure non-limiting availability of electron acceptor. Nitrogen gas sparging was used for mechanical mixing of MBBR carriers and to minimize dissolved oxygen (DO) concentration in the medium.

During long-term operation (approximately 1.5 years) of the two systems, two sets of batch experiments (batch 1: day 100; batch 2: day 471) were performed with biofilm from each MBBR (U, S1, S2, S3). MBBRs were disconnected and experiments were run for 24 h (batch 1) or 49 h (batch 2) using the same initial feeding medium (pre-clarified wastewater from Mølleåværket WWTP and excess nitrate at initial concentration of 104 mgN L^{-1}). Concentration of bulk pollutants and pharmaceuticals were measured at regular intervals during the experiments.

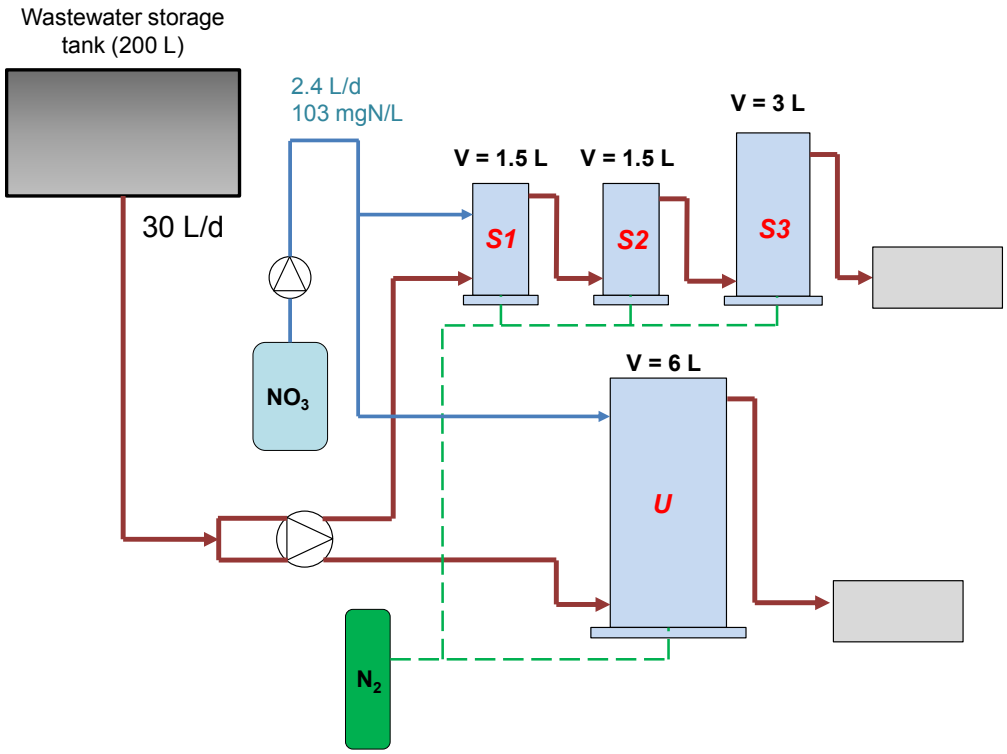


Figure 5.1. Schematic presentation of the single-stage (U) and three-stage (S1, S2, S3) MBBRs.

In this section, results from batch 2 are presented. Batch experiments aimed at characterizing biokinetics of heterotrophic denitrification and pharmaceuticals elimination in each MBBR. Thus, the main goal of our investigation was to assess whether differences in denitrification and pharmaceutical removal capabilities were induced by prolonged biofilm exposure to different electron donor loading/availability conditions. Specifically, we tested two hypotheses:

- A kinetic improvement of pharmaceutical biotransformation occurs under strongly limiting electron donor loading/availability (e.g., in S3) due to, e.g., mixed substrate utilization strategies;

- Electron donor loading/availability, by supporting the growth of denitrifying bacteria, exerts a positive influence on the biotransformation kinetics of pharmaceuticals occurring via cometabolism.

To test these two hypotheses, identification of appropriate model structures describing XTC removal during batch experiments was a crucial step. Through our investigation, we also aimed at assessing XTC biotransformation under denitrifying conditions, for which limited evidences are currently available (Plósz et al., 2010, 2012; Falås et al., 2013; Suarez et al., 2010; Su et al., 2015) as compared to aerobic conditions.

5.1 Modelling removal kinetics in batch experiments

In the batch experiments performed with MBBR biofilm, typical profiles of aqueous XTC concentration were recognized (Fig. 5.2).

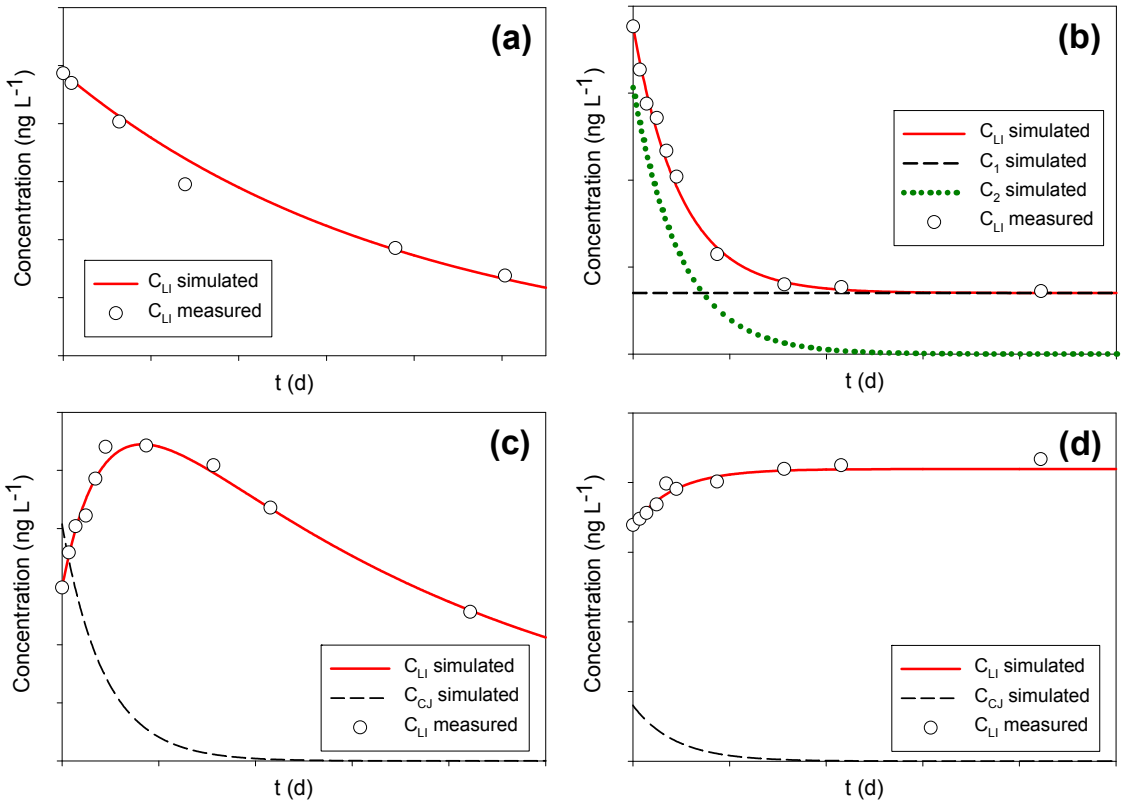


Figure 5.2. Typical profiles of pharmaceutical concentrations during batch experiments (circles) and simulation results with calibrated model (red line): (a) biotransformation only; (b) enantioselective biotransformation; (c), (d) retransformation and biotransformation.

Preliminary model identification, based on the ASM-X framework, was thus required to describe observed profiles. Eventually, rate constants could be estimated by calibrating identified models to measurements and compared among different MBBRs. No effect of primary substrate (inhibition, reductant regeneration) was observed. Pseudo-first-order kinetics was considered with respect to aqueous XTC concentration. Model structures identified are summarized in Table 5.1. Pseudo-first-order biotransformation was sufficient to describe removal kinetics only for a number of XTCs (Fig. 5.2a). Biotransformation of the beta-blocker atenolol resulted from a faster and a slower transformation process (Fig. 5.2b). Stereoselective biotransformation of the two enantiomers of atenolol (C_1, C_2) was hypothesized based on previous experimental findings (Kasprzyk-Hordern and Baker, 2011; Vazquez-Roig et al., 2014). Formation of XTCs (Fig. 5.2c–d) was observed and associated to the retransformation of conjugated XTCs (e.g., sulfonamides, diclofenac; 2.2.3).

Table 5.1. Model structures identified to describe removal kinetics of XTCs during MBBR batch experiments. Equations refer to XTC profiles presented in Figure 5.2.

Case	Processes	States				Rate equation
		C_{LI}	C_1	C_2	C_{CJ}	
Biotransformation only (Fig. 5.2a)	Biotransformation of C_{LI}	-1				$\frac{k_{bio}}{1+K_d X_{SS}} C_{LI} X_{SS}$
Enantioselective biotransformation (Fig. 5.2b)	Biotransformation of enantiomer 1	-1	-1			$\frac{k_{bio,1}}{1+K_d X_{SS}} C_1 X_{SS}$
	Biotransformation of enantiomer 2	-1		-1		$\frac{k_{bio,2}}{1+K_d X_{SS}} C_2 X_{SS}$
Biotransformation and retransformation (Fig. 5.2c, 5.2d)	Biotransformation of C_{LI}	-1				$\frac{k_{bio}}{1+K_d X_{SS}} C_{LI} X_{SS}$
	Formation of C_{LI}	+1 (F)*			-1	$k_{dec} C_{CJ} X_{SS}$

*In case the XTC has only one known retransformable conjugate, the stoichiometry coefficient F should be used, being equivalent to the ratio of the molecular weight of parent (MW_{LI}) and conjugated (MW_{CJ}) XTC.

5.2 Influence of primary metabolism on XTC biotransformation kinetics

By calibrating models presented in Table 5.1 to batch experimental data, it was possible to estimate transformation rate constants (k_{bio}, $k_{bio,1}$, $k_{bio,2}$, k_{dec}) for selected XTCs in S1, S2, S3, U. Biofilm capacity of reducing nitrogen oxides was also assessed by determining the denitrification potential in different MBBRs. The denitrification potential (DNP, mgN $gTSS^{-1}$; Eq. 5.1) was calculated over the duration of batch experiments based on initial and final measured NO_X concentrations (mgN L^{-1}):

$$DNP = (NO_{X,0} - NO_{X,final}) / X_{TSS} \quad (5.1)$$

where X_{TSS} denotes the TSS concentration (gTSS L^{-1}) in MBBR reactors. NO_X included NO_3-N and NO_2-N concentrations to account for nitrite accumulation: (Ubay Çokgör et al., 1998; Eq. 5.2):

$$NO_X = NO_3 - N + 0.6 NO_2 - N \quad (5.2)$$

In Figure 5.3, we present transformation rate constants of non-recalcitrant pharmaceuticals (k_{bio}, k_{dec} > 0.1 L gTSS-1 d-1) as a function of denitrification potential DNP in different MBBRs. Highest and lowest DNP values were observed in S1 and S3, respectively. A strong correlation was shown for the selected XTCs (sulfamethoxazole, erythromycin, trimethoprim; atenolol to a lower extent) between transformation rate constants and DNP in MBBR reactors. These results indicate that the long-term exposure to different COD loading (resulting from reactor staging) and availability (S1 versus U) influenced biofilm capacity of reducing nitrogen oxides and degrading XTCs. More in detail, simultaneous exposure of S1 biofilm to the highest COD loading, heterogeneity and availability may have influenced the metabolic capacity to access a broad range of carbon sources, including pharmaceuticals. Such influence resulted in the observed enhancement of heterotrophic denitrification and transformation kinetics for some of the pharmaceuticals investigated ($k_{bio} \geq 1$ L $gTSS^{-1}$ d^{-1} for sulfamethoxazole, erythromycin, atenolol; Fig. 5.3). The positive correlation showed in Figure 5.3 further demonstrates that XTC biotransformation occurred via cometabolism, where heterotrophic denitrification represented the primary metabolic process supporting microbial growth.

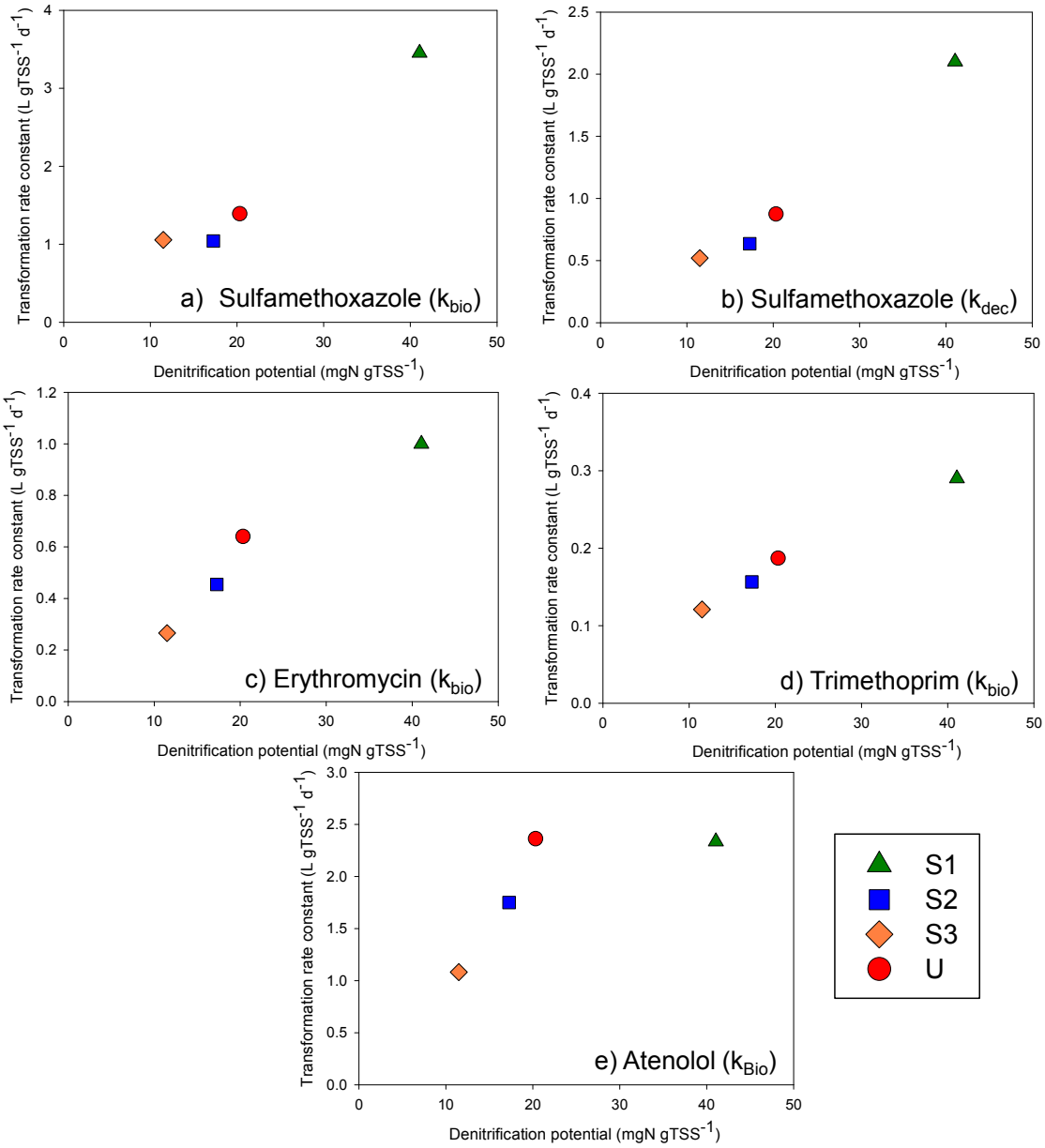


Figure 5.3. Correlation between estimated transformation rate constants (k_{bio}, k_{dec}) and denitrification potential (DNP, Eq. 5.1) during batch experiments in different MBBR reactors. Denitrification potential is here used as an indicator of primary metabolic processes in biofilm.

With respect to the antibiotic sulfamethoxazole, it is worth highlighting that (i) retransformation—most probably from conjugated metabolites—was found significant (Fig. 5.2c; Fig. 5.3b); (ii) k_{bio} values estimated in our study (in particular for S1) were higher than for activated sludge (see Table 1 in **Paper II**; compare with Table S4 in **Paper III**). This may indicate that enhanced sulfamethoxazole elimination may occur in MBBR systems. The significance of these two evidences on sulfamethoxazole elimination in full-scale WWTPs is assessed in detailed in Chapter 6 and in **Paper III**.

6 Factors influencing full-scale XTC removal: the case of sulfamethoxazole

Due to intensive research in the last two decades, a large number of scientific studies has presented measured removal efficiencies for XTCs in full-scale WWTPs. Reported removal efficiencies have been characterized by significant variability for the same substance (see, e.g., review studies by Michael et al., 2013; Luo et al., 2014; see also Fig. 3 in **Paper IV**). Such variability can be attributed both to experimental approaches (e.g., sampling protocols) used in these studies and to differences in XTC fate processes, occurring in full-scale WWTPs.

As outlined in the previous sections, two crucial factors are likely to influence the elimination of XTCs in full-scale WWTPs, namely (i) retransformation processes (2.2.3), leading to the apparent formation of XTCs; and (ii) solid residence time (SRT) of operation, affecting XTC biotransformation capacity (2.2.1). When retransformation processes are relevant (e.g., in the presence of significant concentrations of conjugated metabolites), monitoring the concentration of parent XTCs may be insufficient, resulting in incomplete mass balance of the investigated chemical.

In **Paper III**, we assessed the influence of retransformation processes and SRT on the observed elimination of XTCs during biological treatment in full-scale WWTPs. We extended a recently developed methodology (Plósz et al., 2012) and compared ASM-X predictions and international literature data on XTC removal efficiency.

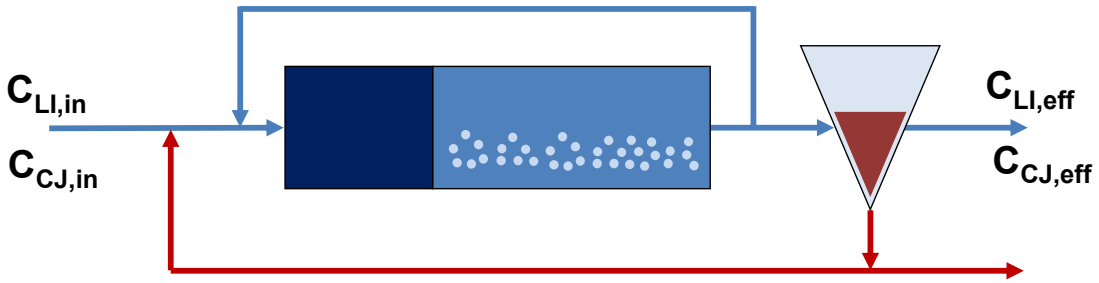


Figure 6.1. Schematic representation of a full-scale biological wastewater treatment configuration, in which XTC removal efficiency was predicted or measured. Removal efficiency calculations were performed based on influent ('in') and effluent ('eff') XTC concentrations of the aqueous parent fraction (C_{LI}) and the retransformable fraction (C_{CJ}).

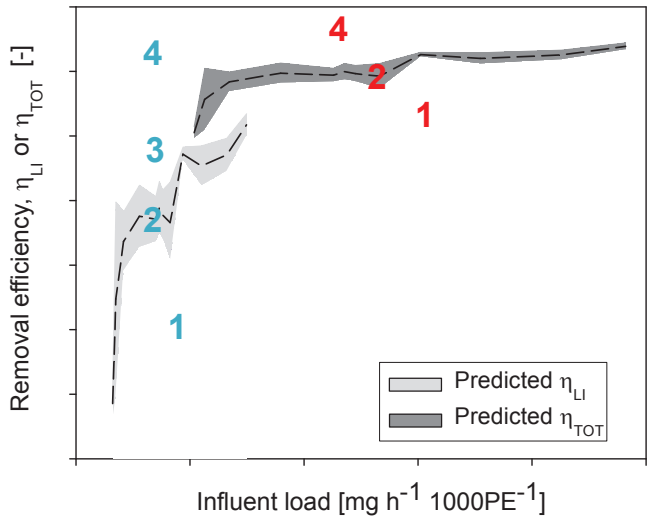
Predicted and measured removal efficiencies were calculated using aqueous parent (C_{LI}) and retransformable (C_{CJ}) XTC concentrations in secondary influent ('*in*') and effluent ('*eff*') (Fig. 6.1). More specifically, we distinguished between (predicted or measured) parent XTC removal efficiency (η_{LI}, Eq. 6.1):

$$\eta_{LI} = \frac{M_{LI,in} - M_{LI,eff}}{M_{LI,in}} \approx \frac{C_{LI,in} - C_{LI,eff}}{C_{LI,in}} \tag{6.1}$$

and total XTC removal efficiency (η_{TOT}, Eq. 6.2), accounting for retransformable XTC fractions:

$$\eta_{TOT} = \frac{M_{LI,in} + M_{CJ,in} - M_{LI,eff} - M_{CJ,eff}}{M_{LI,in} + M_{CJ,in}} \approx \frac{C_{LI,in} + C_{CJ,in} - C_{LI,eff} - C_{CJ,eff}}{C_{LI,in} + C_{CJ,in}} \tag{6.2}$$

where M and C denote XTC mass loads (g d^{-1}) and concentrations (ng L^{-1}), respectively. The impact of retransformation and SRT on XTC elimination was graphically assessed (and quantified) using operating plots as in Figure 6.2. The figure summarizes the possible cases (1–4) arising from the comparison of ASM-X predictions and literature data.



When considering measured η_{LI}:

1-2: Significant retransformation occurring in WWTP

3: Retransformation prevailing in upstream sewer

4: Enhanced removal at extended SRT

When considering measured η_{TOT}:

1-2: Significant retransformation occurring in WWTP

4: Enhanced removal at extended SRT

Figure 6.2. Operating plots presenting ASM-X prediction curves of parent-based (η_{LI}) and total (η_{TOT}) removal efficiency. Numbers refer to the situations arising from the comparison of literature data points with ASM-X predictions (see description on the right).

ASM-X suited the purpose of our assessment as: (i) the only model explicitly describing retransformation of XTCs, apart from estrogens (Table 3.1); (ii) a dynamic model, required to describe XTC fate in full-scale WWTPs (3.2). Importantly, the influence of experimental approaches on the quantification

of XTC elimination was adequately minimized. We performed a selection of literature studies employing appropriate sampling protocols, accounting for XTC loading dynamics in WWTP influent and effluent (Ort et al., 2010a; Majewsky et al., 2011b).

In the following sections, we will present results from our assessment for the antibiotic sulfamethoxazole. Sulfamethoxazole is a widely used antibiotic, excreted in parent form (13%–21% of consumed dose) and as N_4-acetylated (43%–61%) and N_1-glucuronide conjugate (10%–13%; see also Figure 1, **Paper III**). As for other sulfonamide antibiotics, formation of parent sulfamethoxazole due to biotransformation of its acetylated metabolite was experimentally observed in water and wastewater (e.g., Radke et al., 2009; Plósz et al., 2010a).

6.1 Influence of retransformation

Figure 6.3 presents the comparison between η_{LI} and η_{TOT} predicted with ASM-X and measured in a full-scale WWTP, operating at SRT<16 d (Göbel et al., 2005, 2007). Measured η_{TOT} includes the elimination of parent and acetylated sulfamethoxazole (C_{CJ} = concentration of N_4-acetyl-sulfamethoxazole in Eq. 6.2), the only measured metabolite.

ASM-X predictions for both η_{LI} and η_{TOT} were found to be rather close to data reported by Göbel et al. (2005, 2007). Very low elimination or significant formation of sulfamethoxazole (η_{LI} = -107%–9%) were measured in the full-scale WWTP, resulting from the almost complete deconjugation of N_4-acetyl-sulfamethoxazole. The graphical evaluation further indicates that retransformation of sulfamethoxazole occurred mostly in the WWTPs (case 1–2 in Fig. 6.2). Notably, η_{LI} significantly underestimated the removal of sulfamethoxazole, which could be instead more adequately described by η_{TOT}. The underestimation error between η_{LI} and η_{TOT} in measurements (-160%–44%) was close to ASM-X predictions (-200%–60%).

In addition, the good agreement between full-scale measurements and ASM-X predictions for η_{TOT} indicates that the retransformable fraction could be completely described by its N_4-acetylated conjugate. Thus, the N_1-glucuronide conjugate may not be relevant for the fate of sulfamethoxazole in full-scale WWTPs due to: (i) extensive retransformation in upstream sewers, with negligible presence in WWTP influent; or (ii) persistence, and thus limited retransformation, during biological wastewater treatment.

These evidences show the necessity of accounting for retransformable metabolites when determining removal efficiencies of XTCs, which would be otherwise significantly underestimated. The impact of retransformation in full-scale WWTPs, causing this underestimation, was well predicted by ASM-X.

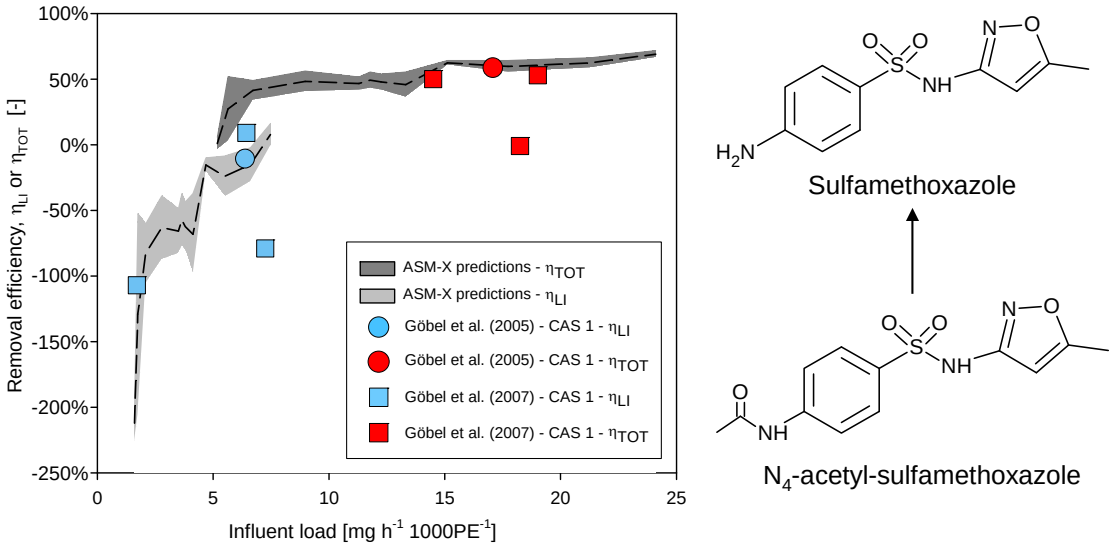


Figure 6.3. Operating plots showing parent-based (η_{LI}) and total removal efficiencies (η_{TOT}) for sulfamethoxazole predicted by ASM-X and measured in full-scale systems (CAS=conventional activated sludge). Measured η_{TOT} includes the elimination of sulfamethoxazole and its acetylated metabolite.

6.1.1 Retransformation: during biological treatment or in upstream sewers?

In Figure 6.3, we showed that significant retransformation may hinder the elimination of parent XTCs—an impact that could be rather well predicted with ASM-X. Conversely, investigations in other WWTPs (Radjenovic et al., 2009; Gurke et al., 2015) reported η_{LI} > 50%, indicating limited retransformation during biological treatment (see Fig. 4a in **Paper III**). In other words, higher removal efficiencies were measured due to significant retransformation occurring upstream to the WWTPs assessed (case 3, Fig. 6.2). It could be thus concluded that processes occurring in upstream sewers may influence the elimination of sulfamethoxazole in WWTPs.

To date, fate in sewer systems (‘reactive sewer’ concept) has been judged relevant for: (i) illicit drugs and their metabolites (e.g., Plósz et al., 2013a; Ramin et al., in preparation); (ii) estrogens and their conjugates (e.g., Gomes et al., 2009; Kumar et al., 2012); and (iii) anti-inflammatory pharmaceuticals (Khan and Ongerth, 2004). To our knowledge, scarce information is available

on XTCs, in particular on antibiotics. Recently, Jelic et al. (2015) observed significant formation of sulfamethoxazole (+ 66%) in pressurized sewers.

Processes occurring in sewer systems likely influence the removal of parent XTCs by changing the WWTP influent composition in terms of parent and retransformable fractions, as compared to the typical composition at the excretion point. Influent composition can be described using the parameter $n_{LI,CJ}$ (Plósz et al., 2010a), defined as the ratio between parent ($C_{LI,in}$) and retransformable XTC fractions ($C_{CJ,in}$) in WWTP influent (Eq. 6.3):

$$n_{LI,CJ} = \frac{C_{LI,in}}{C_{CJ,in}} \tag{6.3}$$

Furthermore, we hypothesized that the residence time in upstream sewers determines the extent of retransformation, causing differences in $n_{LI,CJ}$ among full-scale WWTPs. However, in-sewer residence times are typically not provided in literature studies, making this hypothesis test challenging. Therefore, we assumed that in-sewer residence time may approximated by the design capacity of WWTPs (in population equivalent—PE), giving an indication of the size of a catchment. As a first tentative confirmation, significant retransformation occurred during wastewater treatment in small/medium WWTPs (capacity > 60,000 PE; Göbel at al., 2005; 2007), whereas high η_{LI} > 50%, were observed in large WWTPs (capacity > 250,000 PE; Radjenovic et al., 2009; Gurke et al., 2015).

In Figure 6.4, we present $n_{LI,CJ}$ data in WWTP influents (raw and secondary) as a function of the design capacity of the WWTP. Data from a WWTP treating hospital wastewater (Kovalova et al., 2012) were included to identify a scenario with negligible in-sewer transport. To assess the extent of in-sewer retransformation, measured $n_{LI,CJ}$ were compared with theoretical ratios at the excretion point (Vree et al., 1978; van der Ven et al., 1994). Data from municipal full-scale WWTPs (capacity > 10,000 PE) confirmed our hypothesis, showing relative increase of parent sulfamethoxazole content in influent with increasing WWTP capacity.

Unexpectedly, rather high $n_{LI,CJ}$ were reported for the hospital catchment. This finding may be explained by the excretion of hospitalized patients. Even if pharmaceuticals are administered in hospitals, significant fractions are excreted in households due to reduced hospitalization period (Ort et al., 2010b). In addition, pharmacokinetic studies reported a slower elimination of acetylated sulfamethoxazole from human body than of parent sulfamethoxazole

(Vree and Hekster, 1987; van der Ven et al., 1994; Vree et al., 1995). Altogether, this seems to justify the observation of higher $n_{LI,CJ}$ in hospital sewage.

In summary, retransformation of the acetylated metabolite can significantly influence the observable removal of sulfamethoxazole in full-scale WWTPs. The size and the type of catchment (hospital or municipal) further determine whether retransformation is expected during wastewater treatment or in upstream sewers, justifying the variability of elimination data reported in literature.

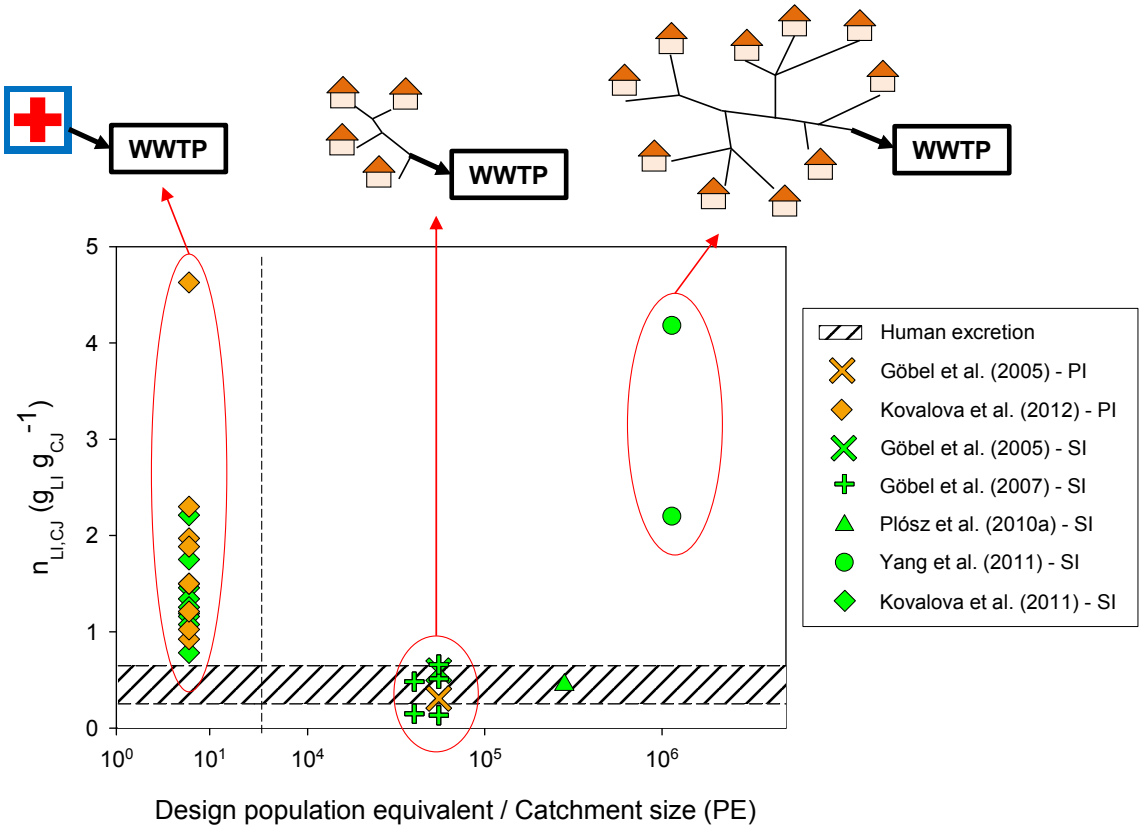


Figure 6.4. Influence of catchment size (indicated by WWTP design capacity) on in-sewer retransformation of conjugates back to parent sulfamethoxazole (PI = primary influent; SI = secondary influent).

6.2 Influence of SRT

Figure 6.5 presents η_{LI} and η_{TOT} predictions and measurements in full-scale WWTPs operating at SRT>16 d, including a biofilm system (fixed bed reactor—FBR). Negative η_{LI} were found (-138% and -61%), possibly resulting from high loads of N_4-acetyl-sulfamethoxazole ($n_{LI,CJ}$=0.15) in secondary influent and thus extensive retransformation.

Nevertheless, these measurements represented an exception, and measured η_{LI} and η_{TOT} were overall significantly higher than in Figure 6.2. Specifically, enhanced elimination was shown in the MBR (η_{LI}=37%-40%; η_{TOT}=70%–90%) under the same loading conditions of CAS 1 (Fig. 6.3). Full-scale data points for η_{LI} and η_{TOT} were accordingly above the respective prediction curves (Fig. 6.5), indicating improved elimination at extended SRT (case 4, Fig. 6.2).

Enhanced biotransformation of sulfamethoxazole at extended SRT was considered to cause these observations, and predictions at k_{bio} = 3 L gTSS^{-1} d^{-1} were used to describe a maximum removal scenario (Fig. 6.5). A step increase of k_{bio} beyond a specific SRT value was assumed to describe enhanced XTC biotransformation according to Plósz et al. (2012), as a result of e.g., enrichment of specialist degrader populations or metabolic expansion. This is in agreement with observations in MBR by Göbel et al. (2007), who found no η_{LI} and η_{TOT} increase with increasing SRT.

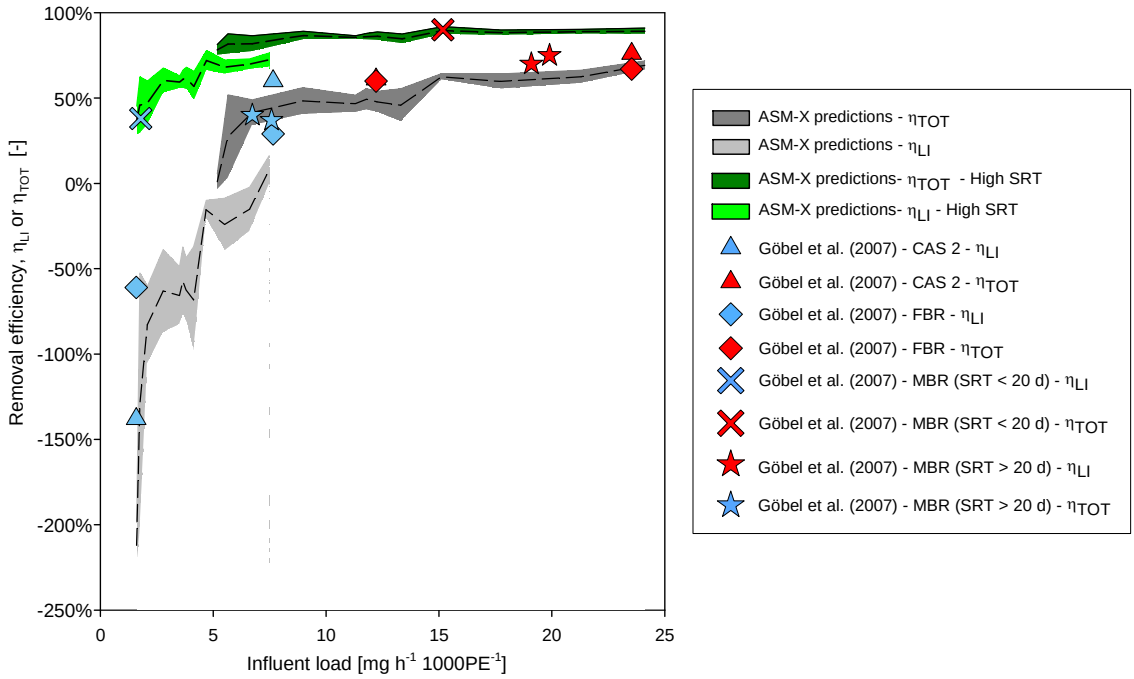


Figure 6.5. Operating plots showing parent-based (η_{LI}) and total removal efficiencies (η_{TOT}) for sulfamethoxazole predicted by ASM-X and measured in full-scale systems. Measured η_{TOT} includes the elimination of sulfamethoxazole and its acetylated metabolite. Scenario simulations to predicted η_{LI} and η_{TOT} at extended SRT ('High SRT' curves) were run assuming k_{bio} = 3 L gTSS^{-1} d^{-1}.

To date, laboratory- and pilot-scale studies have provided for controversial results for systems at extended SRT. In MBR systems, enhancement of

sulfamethoxazole biotransformation was not reported (Abegglen et al., 2009; Fernandez-Fontaina et al., 2013; SRT > 100 d), and improved removal was rather attributed to operation at high TSS concentrations (Sahar et al., 2011). In **Paper II**, conversely, we showed that k_{bio} > 1 L $gTSS^{-1}$ d^{-1} were obtained for sulfamethoxazole in pre-denitrifying MBBR, being significantly higher than rate constants for suspended growth systems (Fig. 5.3; see also Table S4 in **Paper III**).

The presented model-based assessment revealed the possibility for enhanced elimination of sulfamethoxazole in full-scale high SRT WWTPs. Whether this should be associated to a kinetic improvement of biotransformation, it could be proved only for biofilm systems (**Paper II**).

7 Beyond WWTPs: fate of XTCs in agricultural systems

Removal via biodegradation of XTCs in conventional WWTPs is generally incomplete, with consequent release with WWTP effluents and/or accumulation in sewage sludge. Significant XTCs emissions thus occur (i) to freshwater bodies, receiving WWTP effluents; and (ii) to agricultural soils, due to fertilization with sewage sludge. In the European Union, reuse of sewage sludge as fertilizer is a practice encouraged by the 86/278/EEC Directive.

At regulatory level, the risk posed by the environmental release of XTCs has been acknowledged through the application of the regulation (EC) 1907/2006, also known as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). Environmental risk assessment strategies have been accordingly implemented for the prioritization of marketed chemicals. Chemical prioritization is based on prediction of environmental exposure associated toxicity effects in environmental media (e.g., freshwater, agricultural soil).

Besides application of sewage sludge, XTC emissions to agricultural soils occur due to irrigation with freshwater and treated WWTP effluent (Ternes et al., 2007; Calderón-Preciado et al., 2011). Overall, these practices may lead to the uptake and subsequent accumulation of XTCs in food crops, with unclear effects for human health due to dietary intake (Prosser and Sibley, 2015; Malchi et al., 2015). Assessing the fate of XTCs in agricultural systems is a largely uninvestigated subject, particularly in the European Union.

In **Paper IV**, we developed and applied a generic simulation system to predict the fate of ionizable XTCs from consumption/excretion to the accumulation in food crops (namely, winter wheat). Three ionizable XTCs were selected (triclosan, furosemide, ciprofloxacin), for which realistic emission data for different areas in the European Union were used. In the following sections, we will give a brief description of the simulation system and of most relevant predictions in terms of plant uptake.

7.1 A generic fate modelling framework

Three subsequent steps were considered in predicting the fate of XTCs (Fig. 7.1): (i) household consumption and excretion to sewer systems; (ii) elimination in municipal WWTP; and (iii) fate in agricultural systems, following

emissions to soil via fertilization with sewage sludge or irrigation with freshwater. The generic modelling framework combined the WWTP model Activity SimpleTreat (Franco et al., 2011; see 3.1) and the dynamic soil-plant model (Legind et al., 2012; Trapp and Eggen, 2013; see 3.4). A time interval of two consecutive years with fertilization or irrigation was used to simulate XTC uptake into wheat.

Published XTC consumption/emission data and environmental conditions (e.g., precipitation) were used as input for predictions in four different geographical areas of the European Union (Germany, Denmark, Sweden and Italy). Model predictions were eventually validated with measured data—when available—from the geographical areas of the European Union.

Significant variability is often associated to XTC properties used as input in fate models, e.g., excreted fractions (Ort et al., 2009) and biodegradation rates (e.g., Franco and Trapp, 2009). In **Paper IV**, input parameters were derived from literature, and reported parameter variability was accounted for as a source of uncertainty in model predictions. A Monte Carlo-based approach, adapted from Sin et al. (2009), was used to determine the uncertainty propagation to model output.

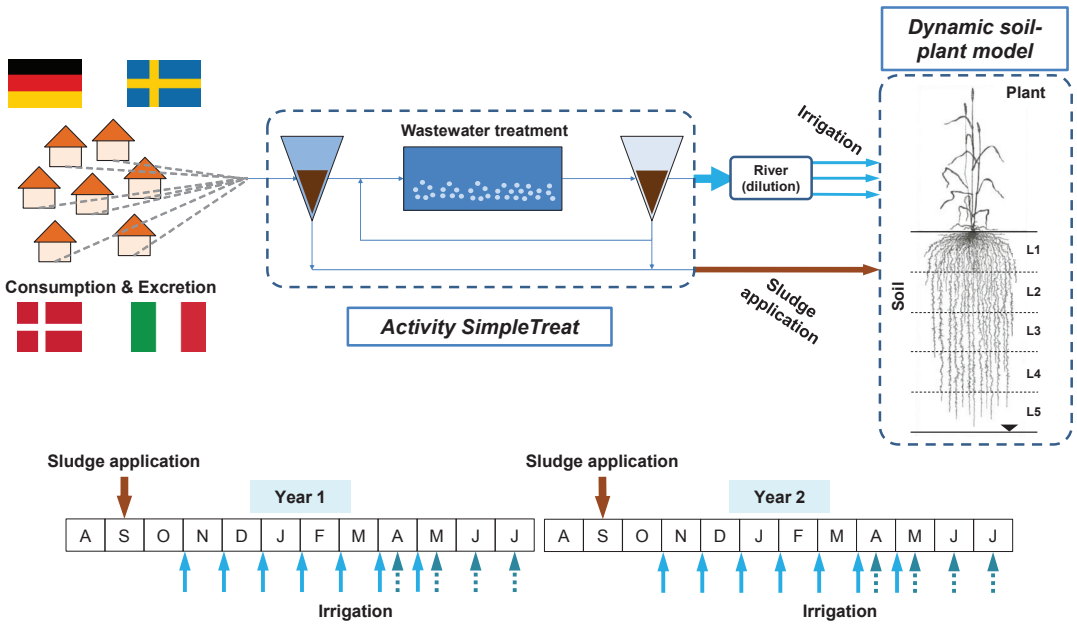


Figure 7.1. System boundaries for fate prediction of TCS, FUR and CIP from consumption to uptake into winter wheat. The time frame for sludge application and irrigation scenarios are reported (full arrows: Italian scenario; dashed arrows: Danish scenario).

Fate predictions for triclosan, furosemide and ciprofloxacin were reported, compared and tentatively validated in Figures 2–5 of **Paper IV**. An example is presented here for furosemide in the Danish scenario (Fig. 7.2). Furosemide is a highly consumed pharmaceutical in Denmark (medstat.dk), with estimated emissions of more than 500 g 1000cap^{-1} a^{-1} to urban WWTPs. Negligible sorption of furosemide to sludge was predicted, due to its low lipophilicity and its speciation as anion at wastewater pH (pK_a=3.34–3.90). Limited WWTP elimination was accordingly shown, resulting in significant emissions to freshwater. A significant fraction of emissions to soil (0.28 ± 0.07 g ha^{-1} a^{-1}) was predicted to be uptaken in winter wheat (48.9%). Significant uncertainty was associated to fractions uptaken in plant compartment (up to 80% relative standard deviation).

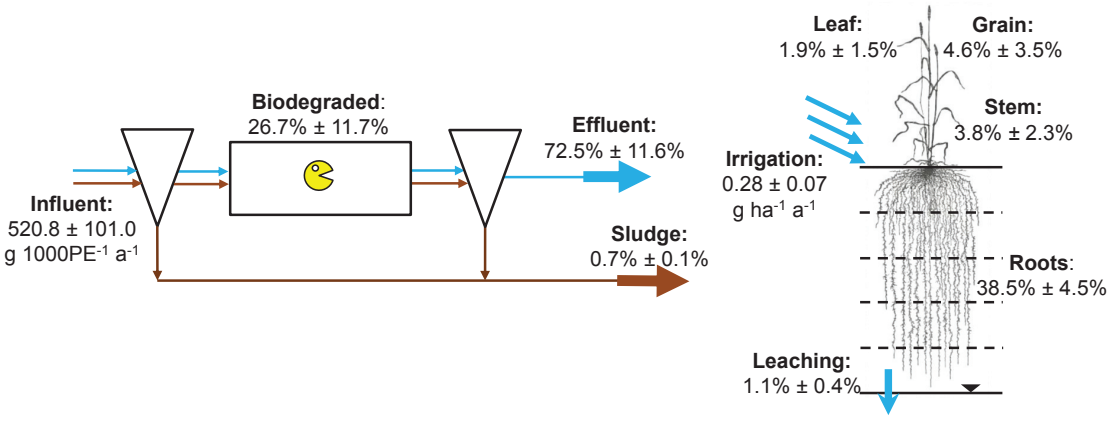


Figure 7.2. Fate prediction in the generic WWTP and agricultural system for furosemide in the Danish scenario, with freshwater irrigation. Input to WWTP and soil-plant system is presented in g 1000cap^{-1} a^{-1} and g ha^{-1} a^{-1}, respectively. Output mass loads in the generic WWTP and in the soil-plant system are presented as % fraction of input load. Model predictions are presented as mean ± standard deviation of 50,000 Monte Carlo simulations.

7.2 Uptake in winter wheat

Model predictions showed significantly different plant uptake for the three ionizable XTCs selected (Fig. 7.3), assuming negligible *in planta* degradation. Limited translocation of triclosan (<1% of emissions to soil) was predicted, resulting from low half-life observed in soil (13–110 d; Xu et al., 2009; Walters et al., 2010). Strong binding to soil matrix determined the negligible plant uptake (<0.1 % of emissions to soil) predicted for ciprofloxacin.

Conversely, major translocation of furosemide (on average, >25% of emissions to soil) was predicted. As a polar chemical (pK_a=3.34–3.90; logK_{ow}=1.90–2.32), furosemide undergoes little partitioning in soil (Franco and Trapp, 2008; Franco et al., 2009) and can be co-transported with water into wheat plants. Most of *in planta* accumulation was shown in wheat roots, resulting from high affinity of furosemide to proteins (log$K_{a,HSA}$>5; ACD/DBP, ilab.acdlabs.com). Notably, the overall fraction accumulated in whole wheat plant was found significantly higher in the irrigation scenario (48.9%), as compared to sludge application (27.6%). Irrigation occurs in proximity to plant harvest, when growing wheat plants take up high amounts of soil pore water, whereas fertilization is assumed to take place in September (Fig. 7.1).

Relevant accumulation in wheat grain was accordingly shown only for furosemide (4.2%–4.6% of emission to soil). Differences between XTCs were further highlighted when calculating bioconcentration factors to wheat grain (BCF_{grain}, $kg_{DW}\ kg_{DW}^{-1}$; Eq. 7.1):

$$BCF_{grain} = C_{grain}(harvest) / C_{soil}(amendment) \quad (7.1)$$

where $C_{soil}(amendment)$ denotes XTC concentration in the top soil layer at sludge amendment or at the last irrigation pulse. Highest BCF_{grain} was predicted for furosemide (< 10 $kg_{DW}\ kg_{DW}^{-1}$), with limited uptake for triclosan and ciprofloxacin (< 0.1 $kg_{DW}\ kg_{DW}^{-1}$). Significant uncertainty (up to 12-fold 5^{th}–95^{th} percentile variation) was associated to BCF_{grain} predictions.

Uptake of furosemide into wheat grain is likely associated to its ionization pattern. At phloem pH (=8), furosemide is present as anion and thus undergoes ion trapping (Trapp, 2004, 2009), with transport via transpiration from stem to grains. Although no empirical confirmation of the uptake of furosemide exists, ion trapping may explain the significant uptake into plants (and fruits) of other polar weak acids, namely herbicides (Trapp, 2009) and anti-inflammatory pharmaceuticals (e.g., naproxen; Goldstein et al., 2014).

Predictions of uptake into food crops, and specifically to edible plant tissues, are highly relevant for the estimation of human dietary intake and consequent exposure to XTCs. Based on modelling results presented here, low-to-negligible risk was associated to the dietary intake of triclosan, furosemide and ciprofloxacin. Nevertheless, our model predictions provide evidence only for wheat grain, and investigations on other highly consumed food crops may be required.

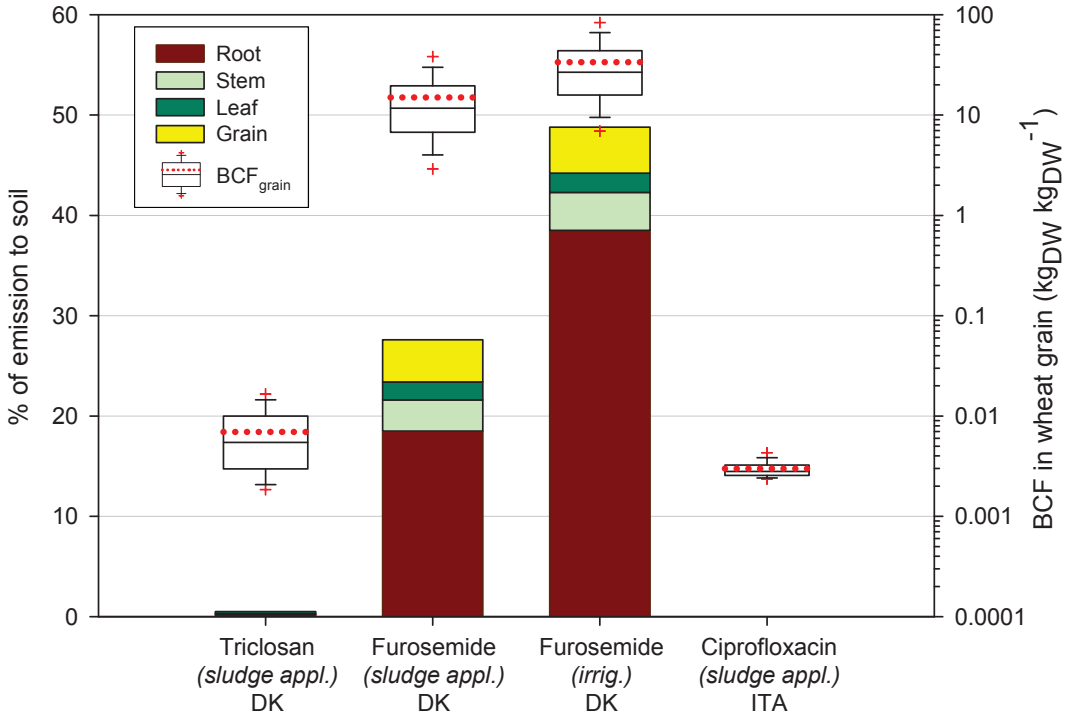


Figure 7.3. Predicted relative translocation to wheat tissues (bars, left y axis) and BCF_{grain} (box plot, right y axis) for triclosan (Denmark—DK, sludge application), furosemide (Denmark—DK, sludge application and irrigation) and ciprofloxacin (Italy—ITA, sludge application). Box plots refer to the uncertainty of simulated model scenarios and include mean values (red dotted lines) and 5th and 95th (red crosses), 10th and 90th (error bars), 25th, 50th and 75th percentiles (boxes).

8 Conclusions

This PhD thesis contributed to improve the understanding of the fate of xenobiotic trace chemicals (XTCs) during and beyond wastewater treatment. Through model-based interpretation of experimental observations, and by developing and testing a number of modelling tools, our research provided for evidences on: (i) the influence of pH and iron salt dosing on the partitioning of zwitterionic XTCs; (ii) the influence of primary metabolism on the biological transformation of XTCs in MBBR biofilm; (iii) the significance of retransformation and solid residence time on the elimination of the antibiotic sulfamethoxazole; and (iv) the potential accumulation in food crops of XTCs released from wastewater treatment plants (WWTPs) via biosolids amendment and freshwater irrigation. To reach the major conclusions of our research, fate modelling was considered from different aspects, namely model development, model identification, model validation and uncertainty analysis.

First, this research showed that pH conditions and, to a lesser extent, iron salt dosing for chemical treatment processes can dramatically impact solid-liquid partitioning of zwitterionic XTCs onto activated sludge. This influence was found substantial for the zwitterionic antibiotic ciprofloxacin, suggesting that partitioning results mainly from electrostatic interactions between charged XTCs and charged sludge surfaces. The highest sorption capacity was shown for ciprofloxacin zwitterion, capable of partitioning via multiple electrostatic interactions (cation exchange, cation bridging, surface complexation). Significant sorption capacity was associated to ciprofloxacin cation, whereas sorption of the anion was negligible due to prevailing Coulombic repulsion with negatively charged sludge surfaces. An extension to traditional partitioning models was proposed to account for XTC ionization with changing pH and different sorption potential of ionized species. Such extension could be beneficial: (i) to predict the partitioning of other XTCs having similar ionization patterns (e.g., fluoroquinolones, tetracyclines), thus ionizing into more than one species at typical sewage pH; (i) when explicitly incorporating pH prediction tools in WWTP models.

Secondly, we investigated biological transformation kinetics of XTCs in predenitrifying MBBRs operated in single-stage and three-stage configurations. Model identification was a crucial step in characterizing biotransformation of indigenous XTCs in real wastewater. Model equations were successfully tested to describe enantioselective biotransformation and retransformation of e.g., XTC conjugates. Based on model-based observations, we demonstrated

that biofilm exposure to different electron donor (i.e., COD for nitrogen oxide reduction) loading and availability conditions influenced XTC biotransformation kinetics in MBBRs. Specifically, denitrifying biofilm developed increasing capacity of biodegrading XTC as a function of COD loading conditions in the three-stage MBBR. Correlation between biotransformation rate constants and denitrification potential was found, indicating that XTC degradation occurred via cometabolism. These evidences provided insights on the fate of XTCs under denitrifying conditions, and support the employment of innovative design concepts to improve XTC elimination during biological wastewater treatment.

Thirdly, by comparing model predictions and literature data, we could demonstrate that the elimination of the antibiotic sulfamethoxazole in full-scale WWTPs is influenced by retransformation of conjugated metabolites and SRT. Through the Activated Sludge Modelling framework for Xenobiotics (ASM-X) it was possible to adequately predict the extent of these impacts. A critical SRT of 16 d was identified to improve the elimination of sulfamethoxazole, possibly confirming our experimental observations of enhanced biotransformation in MBBRs. Importantly, retransformation can prevail in sewers upstream to municipal WWTPs. Therefore, we showed that the catchment size can determine whether retransformation is more significant during secondary wastewater treatment (small catchments) or in upstream sewers (large catchments), thereby influencing the observed removal efficiency in WWTPs. This rule was not applicable to hospital WWTPs ('zero-catchment') due to temporal excretion trends by hospitalized patients. Overall, these findings call for an integrated approach: (i) with respect to XTCs, by evaluating parent and retransformable fractions; (ii) at the system boundary level, by incorporating excretion and in-sewer fate processes in the evaluating XTC removal.

Eventually, a generic modelling tool was developed and tested to predict the fate of ionizable XTCs from consumption and excretion to the uptake in food crops, through the elimination in WWTPs. Among the XTCs selected, the diuretic furosemide was predicted to be particularly recalcitrant to wastewater treatment, in agreement with peer-reviewed literature. As a polar, anionic XTC, furosemide was also predicted to undergo significant accumulation in wheat (30%–50% of emission to soil). Freshwater irrigation enhanced the relative uptake of furosemide in wheat as compared to fertilization with biosolids. This evidence is particularly relevant to the increasingly frequent reuse of reclaimed water for crop irrigation. The developed modelling tool is

intended to be used: (i) for priority setting, e.g., providing estimations of human exposure to XTCs via dietary intake; (ii) to identify experimental research needs, i.e. fate assessment of potentially hazardous XTCs in agricultural systems; and (iii) to predict accumulation in food crops following XTC emissions via alternative pathways, e.g., irrigation with reclaimed wastewater or manure application.

9 References

Abegglen, C., Joss, A., McArdell, C.S., Fink, G., Schlüsener, M.P., Ternes, T.A., Siegrist, H. (2009). The fate of selected micropollutants in a single-house MBR. Water Res. 43, 2036–2046.

Agwuh, K.N., MacGowan, A. (2006). Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J. Antimicrob. Chemother. 58, 256–265.

Alex, J., Benedetti, L., Copp, J., Gernaey, K.V., Jeppsson, U., Nopens, I., Pons, M.N., Rieger, L., Rosen, C., Steyer, J.P., Vanrolleghem, P., Winkler, S. (2008). Benchmark Simulation Model no. 1 (BSM1). Report by IWA Taskgroup on Benchmarking of Control Strategies for WWTPs, Lund University, Sweden.

Alexander, M. (1985). Biodegradation of organic chemicals. Environ. Sci. Technol. 18(2), 106–111.

Alexander, M. (2000). Aging, bioavailability, and overestimation of risk from environmental pollutants. Environ. Sci. Technol. 34, 4259–4265.

Alvarez-Cohen, L., Speitel, Jr., G.E. (2003). Kinetics of aerobic cometabolism of chlorinated solvents. Biodegr. 12, 105–126.

Andersen, H., Siegrist, H., Halling-Sørensen, B., Ternes, T.A. (2003). Fate of estrogens in a municipal sewage treatment plant. Environ. Sci. Technol. 37, 4021–4026

Andersen, H.R., Hansen, M., Kjølholt, J., Stuer-Lauridsen, F., Ternes, T., Halling-Sørensen, B. (2005). Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. Chemosphere 61, 139–146.

Artola-Garicano, E., Borkent, I., Damen, K. Jager, T., Vaes, W.H.J. (2003a). Sorption kinetics and microbial biodegradation activity of hydrophobic chemicals in sewage sludge: Model and measurements based on free concentrations. Environ. Sci. Technol. 37, 116–122.

Artola-Garicano, E., Hermens, J.L.M., Vaes, W.H.J. (2003). Evaluation of Simple Treat 3.0 for two hydrophobic and slowly biodegradable chemicals: polycyclic musks HHCB and AHTN. Water Res. 37, 4377–4384.

Bagnall, J.P., Ito, A., McAdam, E.J., Soares, A., Lester, J.N., Cartmell, E. (2012). Resource dependent biodegradation of estrogens and the role of ammonia oxidising and heterotrophic bacteria. J. Hazard. Mater. 239–240, 56–63.

Bahlmann, A., Brack, W., Schneider, R.J., Krauss, M. (2014). Carbamazepine and its metabolites in wastewater: Analytical pitfalls and occurrence in Germany and Portugal. Water Res. 57, 104–114.

Barret, M., Patureau, D., Latrille, E., Carrère, H. (2010a). A three-compartment model for micropollutants sorption in sludge: Methodological approach and insights. Water Res. 44, 616–624.

Barret, M., Carrère, H., Latrille, E., Wisniewski, C., Patureau, D. (2010b). Micropollutant and sludge characterization for modeling sorption equilibria. Environ. Sci. Technol. 44, 1100–1106.

Batt, A.L., Kim, S., Aga, D.S. (2006). Enhanced biodegradation of iopromide and trimethoprim in nitrifying activated sludge. Environ. Sci. Technol. 40, 7367–7373.

Bernhard, M., Müller, J., Knepper, T.P. (2006). Biodegradation of persistent polar pollutants in wastewater: Comparison of an optimised lab-scale membrane bioreactor and activated sludge treatment. Water Res. 40, 3419–3428.

Besse, J.P., Garric, J. (2008). Human pharmaceuticals in surface waters: implementation of a prioritization methodology and application to the French situation. Toxicol. Lett. 176, 104–123.

Bester, K. (2003). Triclosan in a sewage treatment process—balances and monitoring data. Water Res. 37, 3891–3896.

Birch, R.R. (1991). Prediction of the fate of detergent chemicals during sewage treatment. J. Chem. Technol. Biotechnol. 50, 411–422.

Bollmann, U.E., Tang, C., Eriksson, E., Jönsson, K., Vollertsen, J., Bester, K. (2014). Biocides in urban wastewater treatment plant influent at dry and wet weather: Concentrations, mass flows and possible sources. Water Res. 60, 64–74.

Boxall, A.B.A., Kolpin, D.W., Halling-Sørensen, B., Tolls, J. (2003). Are veterinary medicines causing environmental risks? Environ. Sci. Technol. 37, 286A–294A.

Brown, A.K., Wong, C.S. (2015). Current trends in environmental analysis of human metabolite conjugates of pharmaceuticals. Trends Environ. Anal. Chem. 5, 8–17.

Byrns, G. (2001). The fate of xenobiotic organic compounds in wastewater treatment plants. Water Res. 35, 2523–2533.

Calamari, D., Zuccato, E., Castiglioni, S., Bagnati, R., Fanelli, R. (2003). Strategic survey of therapeutic drugs in the rivers Po and Lambro in Northern Italy. Environ. Sci. Technol. 37, 1241–1248.

Calderón-Preciado, D., Jiménez-Cartagena, C., Matamoros, V., Bayona, J.M. (2011). Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. Water Res. 45, 221–231.

Carballa, M., Omil, F., Lema, J.M. (2005). Removal of cosmetic ingredients and pharmaceuticals in sewage primary treatment. Water Res. 39, 4790–4796.

Carrasquillo, A.J., Bruland, G.L., Mackay, A.A., Vasudevan, D. (2008). Sorption of ciprofloxacin and oxytetracycline zwitterions to soils and soil minerals: influence of compound structure. Environ. Sci. Technol. 42, 7634–7642.

Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., Zuccato, E. (2006). Removal of pharmaceuticals in sewage treatment plants in Italy. Environ. Sci. Technol. 40, 357–363.

Celiz, M.D., Tso, J., Aga, D.S. (2009). Pharmaceutical metabolites in the environment: analytical challenges and ecological risks. Environ. Toxicol. Chem. 28, 2473–2484.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., Kroiss, H. (2005a). The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res. 39, 97–106.

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., Kroiss, H. (2005b). Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Res., 39(19), 4797–4807.

Clark, B., Henry, J.G., MacKay, D. (1995). Fugacity analysis and model of organic chemical fate in a sewage treatment plant.

Cloutier, F., Clouzot, L., Vanrolleghem, P.A. (2012). Predicting the fate of emerging contaminants in wastewater treatment plants. In: Proceedings of WEFTEC 2012, 85th Annual Water Environment Federation Technical Exhibition and Conference, New Orleans, Louisiana, USA, 29 September–3 October 2012, 3828–3836.

Comeau, Y. (2008). Microbial metabolism. In Henze, M., van Loosdrecht, M.C.M., Ekama, G., Brdjanovic, D., "Biological wastewater treatment—principles, modelling and design", IWA Publishing, London, UK.

Coutu, S., Wyrsch, V., Wynn, H.K., Rossi, L., Barry, D.A. (2013). Temporal dynamics of antibiotics in wastewater treatment plant influent. Sci. Total Environ. 458–460, 20–26.

Cowan, C.E., Larson, R.J., Feijtel, T.C.J., Rapaport, R.A. (1993). An improved model for predicting the fate of consumer product chemicals in wastewater treatment plants. Water Res. 27, 561–573.

Cunningham, V.L., D'Aco, V.J., Pfeiffer, D., Anderson, P.D., Buzby, M.E., Hannah, R.E., Jahnke, J., Parke, N.J. (2012). Predicting concentrations of trace organic compounds in municipal wastewater treatment plant sludge and biosolids using the PhATETM model. Integr. Environ. Assess . Manag. 8, 530–542.

Dalkmann, P., Siebe, C., Amelung, W., Schoter, M., Siemens, J. (2014). Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil? Environ. Sci. Technol. 48, 4963–4970.

Daughton, C.G., Ternes, T.A. (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Persp. 107, 907–938.

de Graaff, M.S., Vieno, N.M., Kujawa-Roeleveld, K., Zeeman, G., Temmink, H., Buisman, C.J.N. (2011). Fate of hormones and pharmaceuticals during combined anaerobic treatment and nitrogen removal by partial nitritation-anammox in vacuum collected black water. Water Res. 45, 375–383.

De Keyser, W., Gevaert, V., Verdonck, F., Nopens, I., De Baets, B., Vanrolleghem, P.A., Mikkelsen, P.S., Benedetti, L. (2010). Combining multimedia models with integrated urban water system models for micropollutants. Water Sci. Technol. 62, 1614–1622.

Delgadillo-Mirquez, L., Lardon, L., Steyer, J.P., Patureau, D. (2011). A new dynamic model for bioavailability and cometabolism of micropollutants during anaerobic digestion. Water Res. 45, 4511–4521.

Delle Site, A. (2001). Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review. J. Phys. Cehm. Ref. Data 35, 187–439.

Egli, T. (2010). How to live at very low substrate concentration. Water Res. 44, 4826–4837.

Ekama, G.A., Wentzel, M.C. (2008). Nitrogen removal. In Henze, M., van Loosdrecht, M.C.M., Ekama, G., Brdjanovic, D., "Biological wastewater treatment—principles, modelling and design", IWA Publishing, London, UK.

European Chemicals Agency (2008). Guidance on information requirements and chemical safety assessment. Chapter R.16: Environmental Exposure Estimation. Available at http://guidance.echa. europa.eu/docs

European Commission (2004). European Union System for the Evaluation of Substances 2.0 (EUSES 2.0). Prepared for the European Chemicals Bureau by the National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands (RIVM Report no. 601900005). Available via the European Chemicals Bureau. http://ecb.jrc.it.

European Medicines Agency (2006). Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use. Doc. Ref. EMEA/CHMP/ SWP/4447/00.

Evans, S. E., Davies, P., Lubben, A., Kasprzyk-Hordern, B. (2015) Determination of chiral pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction, solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry. Anal. Chim. Acta 882, 112–126.

Falås, P., Baillon-Dhumez, A., Andersen, H.R., Ledin, A., la Cour Jansen, J. (2012). Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals. Water Res. 46, 1167–1175.

Falås, P., Longrée, P., la Cour Jansen, J., Siegrist, H., Hollender, J., Joss, A. (2013). Micropollutant removal by attached and suspended growth in a hybrid biofilm-activated sludge process. Water Res. 47, 4498–4506.

Fauser, P., Vikelsøe, J., Sørensen, P.B., Carlsen, L. (2003). Phthalates, nonylphenols and LAS in an alternately operated wastewater treatment plant—fate modelling based on measured concentrations in wastewater and sludge. Water Res. 37, 1288–1295.

Fernandez-Fontaina, E., Carballa, M., Omil, F., Lema, J.M. (2014). Modelling cometabolic biotransformation of organic micropollutants in nitrifying reactors. Water Res. 65, 371–383.

Fischer, K., Majewsky, M. (2014). Cometabolic degradation of organic wastewater micropollutants by activated sludge and sludge-inherent microorganisms. Appl. Microbiol. Biotechnol. 98, 6583–6597.

Forrez, I., Carballa, M., Boon, N., Verstraete, W. (2008). Biological removal of 17α-ethinylestradiol (EE2) in an aerated nitrifying fixed bed reactor during ammonium starvation. J. Chem. Technol. Biotechnol. 84, 119–125.

Franco, A., Trapp, S. (2008). Estimation of the soil-water partition coefficient normalized to organic carbon for ionizable organic chemicals. Environ. Toxicol. Chem. 27, 1995–2004.

Franco, A., Fu, W., Trapp, S. (2009). Influence of soil pH on the sorption of ionizable chemicals: modeling advances. Environ. Toxicol. Chem. 28, 458–464.

Franco, A., Trapp, S. (2009). Uncertainty analysis of models for ionizable compounds and examples of regional probabilistic exposure assessment. OSIRIS Deliverable D3.1.11.

Franco, A., Trapp, S. (2010). A multimedia activity model for ionizable compounds: Validation study with 2,4-dichlorophenoxyacetic acid, aniline, and trimethoprim. Environ. Toxicol. Chem. 29, 789–799.

Franco, A., Ferranti, A., Davidsen, C., Trapp, S. (2010). An unexpected challenge: ionizable compounds in the REACH chemical space. Int. J. Life Cycle Assess. 15, 321–325.

Franco, A., Song, L., Trapp, S. (2011). Activity SimpleTreate: User Instructions. Available at: http://homepage.env.dtu.dk/stt

Franco, A., Struijs, J., Gouin, T., Price, O.R. (2013a). Evolution of the sewage treatment plant model SimpleTreat: applicability domain and data requirements. Integr. Environ. Assess. Manag. 9, 560–568.

Franco, A., Struijs, J., Gouin, T., Price, O.R. (2013b). Evolution of the sewage treatment plant model SimpleTreat: use of realistic biodegradability tests in probabilistic model simulations. Integr. Environ. Assess. Manag. 9, 569–579.

Freundlich, H. (1909). Kapillarchemie—Eine Darstellung der Chemie der Kolloid und verwandter Gebiete. Akademische Gesellschaft, Leipzig, Germany.

Gao, L., Shi, Y., Li, W., Niu, W., Liu, J., Cai, Y. (2012). Occurrence of antibiotics in eight sewage treatment plants in Beijing, China. Chemosphere 86, 665–671

García Galán, M.J., Díaz-Cruz, M.S., Barceló, D. (2008). Identification and determination of metabolites and degradation products of sulfonamide antibiotics. Trends Anal. Chem. 27, 1008–1022.

García Galán, M.J., Frömel, T., Müller, J., Peschka, M., Knepper, T., Díaz-Cruz, M.S., Barceló, D. (2012a). Biodegradation studies of N4-acetylsulfapyridine and N4-acetylsulfamethazine in environmental water applying mass spectrometry techniques. Anal. Bioanal. Chem., 402, 2885–2896.

Gaulke, L.S., Strand, S.E., Kalhorn, T.F., Stensel, H.D. (2008). 17α-ethinylestradiol transformation via abiotic nitration in the presence of ammonia oxidizing bacteria. Environ. Sci. Technol. 42, 7622–7627.

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W. (2005). Occurrence and sorption behaviour of sulphonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci Technol., 39, 3981–3989.

Göbel, A., McArdell, C.S., Joss, A., Siegrist, H., Giger, W. (2007). Fate of sulphonamides, macrolides, and trimethoprim in different wastewater technologies. Sci. Total Environ., 372, 361–371.

Goldstein, M., Shenker, M., Chefetz, B. (2014). Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. Environ. Sci. Technol. 48, 5593–5600.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., Giger, W. (2003). Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environ. Sci. Technol., 37, 3243–3249.

Gomes, R.L., Scrimshaw, M.D., Lester, J.N. (2009). Fate of conjugated natural and synthetic steroid estrogens in crude sewage and activated sludge batch studies. Environ. Sci. Technol. 43, 3612–3618.

Grady, Jr., C.P.L., Daigger, G.T., Love, N.G., Filipe, C.D.M. (2011). Biological Wastewater Treatment (3rd edition). IWA Publishing, London, UK.

Grafström, R., Ormstad, K., Moldéus, P., Orrenius, S. (1979). Paracetamol metabolism in the isolated rat liver with further metabolism of a biliary paramcetamol conjugate by the smal intestine. Biochem. Pharmacol. 28, 3573–3579.

Griffith, D.R., Kido Soule, M.C., Matsufuji, H., Eglinton, T.I., Kujawinski, E.B., Gschwend, P.M. (2014). Measuring free, conjugated, and halogenated estrogens in secondary treated wastewater effluent. Environ. Sci. Technol. 48, 2569–2578.

Gruengerich, F.P. (2008). Oxidative, reductive, and hydrolytic metabolism of drugs. In Zhang, D., Zhu, M., Griffith Humphreys, W., "Drug Metabolism in Drug Design and Development", John Wiley & Sons, Inc., Hoboken, New Jersey.

Grung, M., Källqvist, T., Sakshaug, S., Skurtveit, S., Thomas, K.V. (2008). Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. Ecotoxicol. Environ. Saf. 71, 328–340.

Gu, C., Karthikeyan, K.G. (2005). Sorption of the antimicrobial ciprofloxacin to aluminum and hydrous oxides. Environ. Sci. Technol. 39, 9166–9173.

Guellil, A., Boualam, M., Quiquampoix, H., Ginestet, P., Audic, J.M., Block, J.C. (2001). Hydrolysis of wastewater colloidal organic matter by extracellular enzymes extracted from activated sludge flocs. Water Sci. Technol. 43, 33–40.

Gulde, R., Helbling, D.E., Scheidegger, A., Fenner, K. (2014). pH-dependent biotransformation of ionizable organic micropollutants in activated sludge. Environ. Sci. Technol. 48, 13760–13768.

Gurke, R., Rößler, M., Marx, C., Diamond, S., Schubert, S., Oertel, R., Fauler, J. (2015). Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci. Total Environ. 532, 762–770.

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment – A review. Chemosphere 36, 357–393.

Halling-Sørensen, B., Sengeløv, G., Tjørnelund, J. (2002). Toxicity of tetracyclines and teracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch. Environ. Contam. Toxicol. 42, 263–271.

Henze, M., Grady Jr., C.P.L., Gujer, W., Marais, G.v.R., Matsuo, T. (1987) Activated Sludge Model No. 1. IAWQ Scientific and Technical Report, London, UK.

Henze, M., Gujer, W., Mino, T., van Loosdrecht, M. (2000). Activated Sludge Models ASM1, ASM2, ASM2d and ASM3. IWA Publishing, London, UK.

Helbling, D.E., Hollender, J., Kohler, H.P.E., Fenner, K. (2010). Structure-based interpretation of biotransformation pathways of amide-containing compounds in sludge-seeded bioreactors. Environ. Sci. Technol. 44, 6628–6635.

Helbling, D.E., Johnson, D.R., Honti, M., Fenner, K. (2012). Micropollutant biotransformation kinetics associate with WWTP process parameters and microbial community characteristics. Environ. Sci. Technol. 46, 10579–10588.

Helbling, D.E., Johnson, D.R., Lee, T.K., Scheidegger, A., Fenner, K. (2015). A framework for establishing predictive relationships between specific bacterial 16S rRNA sequence abundances and biotransformation rates. Water Res. 70, 471–484.

Holbrook, R.D., Love, N.G., Novak, J.T. (2004). Sorption of 17β-estradiol and 17α-ethinylestradiol by colloidal organic carbon derived from biological wastewater treatment systems. Environ. Sci. Technol. 38, 3322–3329.

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., la Cour Jansen, J., Andersen, H.R. (2011). Determination of sorption of seventy-five pharmaceuticals in sewage sludge. Water Res. 45, 4470–4482.

Hyland, K.C., Dickenson, E.R.V., Drewes, J.E., Higgins, C.P. (2012). Sorption of ionized and neutral emerging trace organic compounds onto activated sludge from different wastewater treatment configurations. Water Res. 46, 1958–1968.

Ihssen, J., Egli, T. (2005). Global physiological analysis of carbon- and energy-limited growing Escherichia coli confirms a high degree of catabolic flexibility and preparedness for mixed substrate utilization. Environ. Microbiol. 7, 1568–1581.

Jelic, A., Rodriguez-Mozaz, S., Barcelo, D., Gutierrez, O. (2015). Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Res. 68, 98–108.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P. (1998). Widespread sexual disruption in wild fish. Environ. Sci. Technol. 32, 2498–2506.

Johnson, A.C., Williams, R.J. (2004). A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works (2004). Environ. Sci. Technol. 38, 3649–3658.

Johnson, A.C., Williams, R.J. (2004). A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. Environ. Sci. Technol., 2004, 38(13), 3649–3658.

Johnson, A.C., Ternes, T., Williams, R.J., Sumpter, J.P. (2008). Assessing the concentrations of polar organic microcontaminants from point sources in the aquatic environment: measure or model? Environ. Sci. Technol. 42, 5390–5399.

Johnson, A.C., Dumont, E., Williams, R.J., Oldenkamp, R., Cisowska, I., Sumpter, J.P. (2013). Do concentrations of ethinylestradiol, estradiol, and diclofenac in European rivers exceed proposed EU environmental quality standards? Environ. Sci. Technol. 47, 12297–12304.

Johnson, D.R., Helbling, D.E., Lee, T.K., Park, J., Fenner, K., Kohler, H.P.E., Ackermann, M. (2015). Association of biodiversity with the rates of micropollutant biotransformations among full-scale wastewater treatment plant communities. Appl. Environ. Microbiol. 81, 666–675.

Joss, A., Andersen, H., Ternes, T., Richle, P.R., Siegrist, H. (2004). Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: Consequences for plant optimization. Environ. Sci. Technol. 38, 3047–3055.

Joss, A., Keller, E., Alder, A.C., Göbel, A., McArdell, C.S., Ternes, T., Siegrist, H. (2005). Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Res., 39(14), 3139–3152.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H. (2006a). Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Res. 40, 1686–1696.

Joss, A., Carballa, M., Kreuzinger, N., Siegrist, H., Zabczynski, S. (2006b). Wastewater treatment. In Joss, A., Ternes, T.A. (eds.), “Human Pharmaceuticals, Hormones and Fragrances—The challenge of micropollutants in urban water management”, IWA Publishing, London, UK.

Karickhoff, S.W., Brown, D.S., Scott, T.A. (1979). Sorption of hydrophobic pollutants on natural sediments. Water Res. 13, 241–248.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J. (2009). The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380.

Kasprzyk-Hordern, B., Baker, D.R. (2011). Enantiomeric profiling of chiral drugs in wastewater and receiving waters. Environ. Sci. Technol. 46, 1681–1691.

Keller, V. (2006). Risk assessment of "down-the-drain" chemicals: search for a suitable model. Sci. Total Environ. 360, 305–318.

Khan, S.J., Ongerth, J.E. (2004). Modelling of pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations. Chemosphere 54, 355–367.

Khunjar, W.O., Love, N.G. (2011). Sorption of carbamazepine, 17α-ethinylestradiol, iopromide and trimethoprim to biomass involves interactions with exocellular polymeric substances. Chemosphere 82, 917–922.

Khunjar, W.O., Mackintosh, S.A., Skotnicka-Pitak, J., Baik, S., Aga, D.S., Love, N.G. (2011). Elucidating the relative roles of ammonia oxidizing and heterotrophic bacteria during the biotransformation of 17α-ethinylestradiol and trimethoprim. Environ. Sci. Technol. 45, 3605–3612.

Kim, S., Eichhorn, P., Jensen, J.N., Weber, A.S., Aga, D.S. (2005). Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. Environ. Sci. Technol. 39, 5816–5823.

Koh, Y.K.K., Chiu, T.Y., Boobis, A.R., Scrimshaw, M.D., Bagnall, J.P., Soares, A., Pollard, S., Cartmell, E., Lester, J.D. (2009). Influence of operating parameters on the biodegradation of steroid estrogens and nonylphenolic compounds during biological wastewater treatment. Environ. Sci. Technol. 43, 6646–6654.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.B. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environ. Sci. Technol. 36, 1202–1211.

Komossa, D., Langebartels, C., Sandermann Jr., H. (1995). Metabolic processes for organic chemicals in plants. In: Trapp, S., McFarlane, J.C. (Eds.), Plant Contamination: Modeling and Simulation of Organic Chemical Processes. CRC Press, Boca Raton, Florida.

Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., McArdell, C.S. (2012). Hospital wastewater treatment by membrane bioreactor: performance and efficiency for organic micropollutant elimination. Environ. Sci. Technol. 46, 1536–1545.

Kumar, V., Johnson, A.J., Nakada, N., Yamashita, N., Tanaka, H. (2012). De-conjugation behavior of conjugated estrogens in the raw sewage, activated sludge and river water. J. Hazard. Mat. 227–228, 49–54.

Kümmerer, K., Henninger, A. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin. Microbiol. Infect. 9, 1203–1214.

Kümmerer, K. (2009a). Antibiotics in the aquatic environment – A review – Part I. Chemosphere 75, 417–434.

Larsen, T.A., Harremoës, P. (1994). Degradation mechanisms of colloidal organic matter in biofilm reactors. Water Res. 28, 1443–1452.

Lee, H.J., Lee, E., Yoon, S.H., Chang H.R., Kim, K., Kwon, J.H. (2012). Enzymatic and microbial transformation assays for the evaluation of the environmental fate of diclofenac and its metabolites. Chemosphere 87, 969–974.

Legind, C.N., Trapp, S. (2009). Modeling the exposure of children and adults via diet to chemicals in the environment with crop-specific models. Environ. Pollut. 157, 778–785.

Legind, C.N., Kennedy, C.M., Rein, A., Snyder, N., Trapp, S. (2011). Dynamic plant uptake model applied for drip irrigation of an insecticide to pepper fruit plants. Pest Manag. Sci. 67 (5), 521–527.

Legind, C.N., Rein, A., Serre, J., Brochier, V., Haudin, C.S., Cambier, P., Houot, S., Trapp, S. (2012). Simultaneous simulations of uptake in plants and leaching to groundwater of cadmium and lead for arable land amended with compost or farmyard manure. PLoS One 7 (10), e47002.

Le-Minh, N., Khan, S.J., Drewes, J.E., Stuetz, R.M. (2010). Fate of antibiotics during municipal water recycling treatment processes. Water Res. 44, 4295–4323.

Lewis, G. N. (1901) The law of physico-chemical change. P. Am. Acad. Arts Sci., 37, 49.

Lewis, G. N. (1907). Outlines of a new system of thermodynamic chemistry. P. Am. Acad. Arts Sci. 43, 259–297.

Li, B., Zhang, T. (2010). Biodegradation and adsorption of antibiotics in the activated sludge process. Environ. Sci. Technol. 44, 3468–3473.

Lindberg, R.H., Olofsson, U., Rendahl, P., Johansson, M.I., Tysklind, M., Andersson, B.A.V (2006). Behavior of fluoroquinolones and trimethoprim during mechanical, chemical, and active sludge treatment of sewage water and digestion of sludge. Environ. Sci. Technol. 40, 1042–1048.

Lindblom, E., Gernaey, K.V., Henze, M., Mikkelsen, P.S. (2006). Integrated modelling of two xenobiotic organic compounds. Water Sci. Technol. 54, 213–221.

Lindblom, E., Press-Kristensen, K., Vanrolleghem, P.A., Mikkelsen, P.S., Henze, M. (2009). Dynamic experiments with high bisphenol-A concentrations modelled with an ASM model extended to include a separate XOC degrading microorganism. Water Res. 43, 3169–3176.

Liu, L., Binning, P.J., Smets, B.F. (2014). Evaluate alternating biokinetic models for trace pollutant cometabolism. Environ. Sci. Technol. 49, 2230–2236.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641.

Macherius, A., Eggen, T., Lorenz, W.G., Reentsma, T., Winkler, U., Moeder, M. (2012). Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. J. Agric. Food Chem. 60, 7785–7791.

Mackay, D. (1979). Finding fugacity feasible. Environ. Sci. Technol. 1218–1223.

Mackay, D., Paterson, S. (1981). Calculating fugacity. Environ. Sci. Technol. 1006–1014.

Mackay, D., Paterson, S. (1982). Fugacity revisited. Environ. Sci. Technol. 654A–660A.

MacKay, A.A., Canterbury, B. (2005). Oxytetracycline sorption to organic matter by metal-bridging. J. Environ. Qual. 34, 1964–1971.

MacKay, A.A., Vasudevan, D. (2012). Polyfunctional ionogenic compound sorption: challenges and new approaches to advance predictive models. Environ. Sci. Technol. 46, 9209–9223.

Majewsky, M., Gallé, T., Yargeau, V., Fischer, K. (2011a). Active heterotrophic biomass and sludge retention time (SRT) as determining factors for biodegradation kinetics of pharmaceuticals in activated sludge. Biores. Technol. 102, 7415–7421.

Majewsky, M., Gallè, T., Bayerle, M., Goel, R., Fischer, K., Vanrolleghem, P.A. (2011b). Xenobiotic removal efficiencies in wastewater treatment plants: Residence time distributions as a guiding principle for sampling strategies. Water Res. 45, 6152–6162.

Manallack, D.T. (2009). The acid–base profile of a contemporary set of drugs: implications for drug discovery. SAR QSAR Environ. Res. 20, 611–655.

Marx, C., Mühlbauer, V., Schubert, S., Oertel, R., Ahnert, M., Krebs, P., Kuehn, V. (2015). Representative input load of antibiotics to WWTPs: predictive accuracy and determination of a required sampling quantity. Water Res. 76, 19–32.

Maurer, M., Escher, B.I., Richle, P., Schaffner, C., Alder, A.C. (2007). Elimination of β-blockers in sewage treatment plants. Water Res. 41, 1614–1622.

Mazioti, A.A., Stasinakis, A.S., Gatidou, G., Thomaidis, N.S., Andersen, H.R. (2015). Sorption and biodegradation of selected benzotriazoles and hydroxybenzothiazole in activated sludge and estimation of their fate during wastewater treatment. Chemosphere 131, 117–123.

McAdam, E., Bagnall, J.P., Koh, Y.K.K., Chiu, T.Y., Pollard, S., Scrimshaw, M.D., Lester, J.N., Cartmell, E. (2010). Removal of steroid estrogens in carbonaceous and nitrifying activated sludge processes. Chemosphere 81, 1–6.

McCormick, J.R.D., Fox, S.M., Smith. L.L., Bitler, B.A., Reichental, J., Origoni, V.E., Muller, W.H., Winterbottom, R., Doerschuk, A.P. (1957). Studies on the reversible epimerization occurring in the tetracycline family. The preparation, properties and proof of structure of some 4-epi-tetracyclines. J. Am. Chem. Soc. 79, 2849–2858.

Melcer, H., Bell, J.P., Thompson, D.J., Yendt, C.M., Kemp, J., Steel, P. (1994). Modeling volatile organic contaminants' fate in wastewater treatment plants. J. Environ. Eng. 120, 588–609.

Miao, X.S., Yang, J.J., Metcalfe, C.D. (2005). Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant. Environ. Sci. Technol. 39, 7469–7475.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. Water Res. 47, 957–995.

Monteith, H.D., Parker, W.J., Bell, J.P., Melcer, H. (1995). Modeling the fate of pesticides in municipal wastewater treatment. Water Environ. Res. 67, 964–970.

Morasch, B., Bonvin, F., Reiser, H., Grandjean, D., de Alencastro, L.F., Perazzolo, C., Chèvre, N., Kohn, T. (2010). Occurrence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part II: Micropollutant removal between wastewater and drinking water. Environ. Toxicol. Chem. 29, 1658–1668.

Namkung, E., Rittmann, B.E. (1987). Estimating volatile organic compound emissions from publicly owned treatment works. J. Water Pollut. Contr. Fed. 59, 670–678.

Nowara, A., Burhenne, J., Spiteller, M. (1997). Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. J. Agric. Food Chem. 45, 1459–1463.

Nyholm, N., Berg, U.T., Ingerslev, F. (1996). Activated sludge biodegradability simulation test. Environmental project 337, The Danish Environmental Protection Agency.

Ødegaard, H., Rusten, B., Westrum, T. (1994). A new moving bed bofilm reactor—Applications and results. Water Sci. Tech., 29, 157–165.

Ødegaard, H. (2006). Innovations in wastewater treatment: the moving bed biofilm process. Water Sci. Technol. 53, 17–33.

Oertel, R., Schubert, S., Mühlbauer, V., Büttner, B., Marx, C., Kirch, W. (2014). Determination of clindamycin and its metabolite clindamycin sulfoxide in diverse sewage samples. Environ. Sci. Pollut. Res. 21, 11764–11769.

Orozco, J.H.G. (2008). Toxicity. In Henze, M., van Loosdrecht, M.C.M., Ekama, G., Brdjanovic, D., "Biological wastewater treatment—principles, modelling and design", IWA Publishing, London, UK.

Ort, C., Gujer, W. (2006). Sampling for representative micropollutant loads in sewer systems. Water Sci. Technol. 54, 169–176.

Ort, C., Hollender, J., Schaerer, M., Siegrist, H. (2009). Model-based evaluation of reduction strategies for micropollutants from wastewater treatment plants in complex river networks. Environ. Sci. Technol. 43, 3214–3220.

Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A. (2010a) Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. Environ. Sci. Technol., 44, 6024–6035.

Ort, C., Lawrence, M.G., Reungoat, J., Eaglesham, G., Carter, S., Keller, J. (2010b). Determining the fraction of pharmaceutical residues in wastewater originating from hospitals. Water Res. 44, 605–615.

Ottmar, K.J., Colosi, L.M., Smith, J.A. (2012). Fate and transport of atorvastatin and simvastatin drugs during conventional wastewater treatment. Chemosphere 88, 1184–1189.

Oulton, R.L., Kohn, T., Cwiertny, D.M. (2010). Pharmaceuticals and personal care products in effluent matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management. J. Environ. Monit. 12, 1956–1978.

Pagliara, A., Carrupt, P.A., Caron, G., Gaillard, P., Testa, B. (1997). Lipophilicity profiles of ampholytes. Chem. Rev. 97, 3385–3400.

Pan, B., Qju, M., Wu, M., Zhang, D., Peng, H., Wu, D., Xing, B. (2012). The opposite impacts of Cu and Mg cations on dissolved organic matter-ofloxacin interaction. Environ. Pollut. 161, 76–82.

Petrie, B., McAdam, E.J., Hassard, F., Stephenson, T. Lester, J.N., Cartmell, E. (2014a). Diagnostic investigation of steroid estrogen removal by activated sludge at varying solids retention time. Chemosphere 113, 101–108.

Petrie, B., McAdam, E.J., Lester, J.N., Cartmell, E. (2014b). Assessing potential modifications to the activated sludge process to improve simultaneous removal of a diverse range of micropollutants. Water Res. 62, 182–190.

Petrie, B., Barden, R., Kasprzyk-Hordern, B. (2015). A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Res. 72, 3–27.

Pignatello, J.P., Xing, B. (1996). Mechanisms of slow sorption of organic chemicals to natural particles. Environ. Sci. Technol. 30, 1–11

Plósz, B.G. (2007). Optimization of the activated sludge anoxic reactor configuration as a means to control nutrient removal kinetically. Water Res. 41, 1763–1773.

Plósz, B.G., Leknes, H., Thomas, K.V. (2010a). Impacts of competitive inhibition, parent compound formation and partitioning behaviour on antibiotic micro-pollutants removal in activated sludge. Environ. Sci. Technol., 44, 734–742.

Plósz, B.G., Leknes, H., Liltved, H., Thomas, K.V. (2010b). Diurnal variations in the occurrence and the fate of hormones and antibiotics in activated sludge wastewater treatment in Oslo, Norway. Sci. Total Environ., 408, 1915–1924.

Plósz, B.G., Langford, K.H., Thomas, K.V. (2012). An activated sludge model for trace xenobiotic chemicals (ASM-X): Assessment of diclofenac and carbamazepine. Biotechnol. Bioeng., 109, 2757–2769.

Plósz, B.G., Vogelsang, C. (2012). Innovative and integrated technologies for the treatment of industrial wastewater—The BIOZO process (Innowatech). Edited by Lopez, A., Di Laconi, C., Mascolo, G., Pollice, A. European Water Research Series, IWA Publishing, London, UK, 91–177.

Plósz, B.G., Reid, M.J, Borup, M., Langford, K.H., Thomas, K.V. (2013a). Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. Water Res., 47, 2129–2140.

Plósz, B.G., Polesel, F., Langford, K.H., Thomas, K.V. (2013b). Generalisation of the activated sludge model for xenobiotic trace chemicals, ASM-X – development of a decision support tool. Abstract presented at the Micropol & Ecohazard 2013, 8th IWA Specialized Conference on "Assessment and control of micropollutants and hazardous substances in water", Zurich, 16–20 June 2013.

Pomiés, M., Choubert, J.M., Wisniewski, C., Miége, C., Budzinski, H., Coquery, M. (2015). Lab-scale experimental strategy for determining micropollutant partition coefficient and biodegradation constants in activated sludge. Environ. Sci. Pollut. Res. 22, 4383–4395.

Prosser, R.S., Lissemore, L., Topp, E., Sibley, P.K. (2014a). Bioaccumulation of triclosan and triclocarban in plants grown in soils amended with municipal dewatered biosolids. Environ. Toxicol. Chem. 33 (5), 975–984.

Prosser, R.S., Trapp, S., Sibley, P.K. (2014b). Modeling uptake of selected pharmaceuticals and personal care products into food crops from biosolids-amended soil. Environ. Sci. Technol. 48, 11397–11404.

Prosser, R.S., Sibley, P.K. (2015). Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. Environ. Int. 75, 223–233.

Radjenovic, J., Pérez, S., Petrovic, M., Barceló, D. (2008). Identification and structural characterization of biodegradation products of atenolol and glibenclamide by liquid chromatography coupled to hybrid quadrupole time-of-flight and quadrupole ion trap mass spectrometry. J. Chromat. A 1210, 142–153.

Radjenovic, J., Petrovic, M., Barceló, D. (2009). Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res., 43(3), 831–841.

Radke, M., Lauwigi, C., Heinkele, G., Mürdter, T.E., Letzel, M. (2009). Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. Environ. Sci. Technol. 43, 3135–3141.

Ramin, P., Libonati, A., Polesel, F., Causanilles Llanes, A., Emke, E., de Voogt, P., Plósz, B.G. Sorption and transformation of illicit drug biomarkers in sewer systems: the role of suspended solids in raw wastewater. In preparation.

Rein, A., Legind, C.N., Trapp, S. (2011). New concepts for dynamic plant uptake models. SAR QSAR Environ. Res. 22, 191–215.

Remmers, E.G., Sieger, G.M., Doerschuk A.P. (1963). Some observations on the kinetics of the C-4 epimerization of tetracycline. J. Pharm. Sci. 52, 752–756.

Rendal, C., Kusk, K.O., Trapp, S. (2011). Optimal choice of pH for toxicity and bioaccumulation studies of ionizing organic chemicals. Environ. Toxicol. Chem. 30, 2395–2406.

Richardson, M.L., Bowron, J.M. (1985). The fate of pharmaceutical chemicals in the aquatic environment. J. Pharm. Pharmacol. 37, 1–12.

Rittmann, B.E. (1992). Microbiological detoxification of hazardous organic contaminants: The crucial role of substrate interactions. Water Sci. Technol. 25, 403–410.

Rubirola, A., Llorca, M., Rodriguez-Mozaz, S., Casas, N., Rodriguez-Roda, I., Barceló, D., Buttiglieri, G. (2014). Characterization of metoprolol biodegradation and its transformation products generated in activated sludge batch experiments and in full scale WWTPs. Water Res. 63, 21–32.

Rusten, B., Hellström, B.G., Hellström, F., Sehested, O., Skjelfoss, E., Svendsen, B. (2006). Pilot testing and preliminary design of moving bed biofilm reactors for nitrogen removal at the FREVAR wastewater treatment plant. Water Sci. Technol.

Sabljic, A, Günsten, H, Verhaar, H, Hermens, H. (1995). QSAR modeling of soil sorption. Improvements and systematic of log Koc vs. log Kow correlations. Chemosphere 31, 4489–4514.

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R., Topp, E. (2012). Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. Sci. Total Environ. 431, 233¬–236.

Sathyamoorthy, S., Chandran, K., Ramsburg, C.A. (2013). Biodegradation and cometabolic modeling of selected beta blockers during ammonia oxidation. Environ. Sci. Technol. 47, 12835–12843.

Sato, T., Qadir, M., Yamamoto, S., Endo, T., Zahoor, A. (2013). Global, regional, and country level need for data on wastewater generation, treatment, and use. Agric. Water Manag. 130, 1–13.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M. (2003). Environmental Organic Chemistry (2nd edition). John Wiley & Sons, Hoboken, New Jersey, USA.

Scuras, S.E., Jobbagy, A., Grady Jr., C.P.L. (2001). Optimization of activated sludge reactor configuration: kinetic considerations. Water Res. 35, 4277–4284.

Sengeløv, G., Halling-Sørensen, B., Aarestrup, F. (2003). Susceptibility of Escherichia coli and Enterococcus faecium isolated from pigs and broiler chickens to tetracycline degradation products and distribution of tetracycline resistance determinants in E. coli from food animals. Vet. Microbiol. 95, 91–101.

Senta, I., Terzic, S., Ahel, M. (2013). Occurrence and fate of dissolved and particulate antimicrobials in municipal wastewater treatment. Water Res., 47(2), 705–714.

Senta, I., Krizmann, I., Ahel, M., Terzic, S. (2014). Assessment of stability of drug biomarkers in municipal wastewater as a factor influencing the estimation of drug consumption using sewage epidemiology. Sci.Total Environ. 487, 659–665.

Seth, R., Webster, E., Mackay, D. (2008). Continued development of a mass balance model of chemical fate in a sewage treatment plant. Water Res. 42, 595–604.

Shi, J., Fujisawa, S., Nakai, S., Mosomi, M. (2004). Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium Nitrosomonas europaea. Water Res. 38, 2323–2330.

Siegrist, H., Alder, A., Gujer, W., Giger, W. (1989). Behaviour and modelling of NTA degradation in activated sludge systems. Water Sci. Technol. 21, 315–324.

Siegrist, H., Joss, A., Alder, A., McArdell-Bürgisser, C., Göbel, A., Keller, E., Ternes, T.A. (2003). Micropollutants—new challenge in wastewater disposal? EAWAG News 57, 7–10.

Sin, G., Gernaey, K.V., Neumann, M.B., van Loosdrecht, M.C.M., Gujer, W., (2009). Uncertainty analysis in WWTP model applications: a critical discussion using an example from design. Water Res. 43, 2894–2906.

Singer, H., Müller, S., Tixier, C., Pillonel, L. (2002). Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments. Environ. Sci. Technol. 36, 4998–5004.

Snip, L.J.P., Flores-Alsina, X., Plósz, B.G., Jeppsson, U., Gernaey, K.V. (2014). Modelling the occurrence, transport and fate of pharmaceuticals in wastewater systems. Environ. Modell. Softw. 62, 112–127.

Sobeck, D.C., Higgins, M.J. (2002). Examination of three theories for mechanisms of cation-induced bioflocculation. Water Res. 36, 527–538.

Stadler, L.B., Ernstoff, A.S., Aga, D.S., Love, N.G. (2012). Micropollutant fate in wastewater treatment: redefining “removal”. Environ. Sci. Technol. 46, 10485−10486.

Stadler, L.B., Su, L., Moline, C.J., Ernstoff, A.S., Aga, D.S., Love, N.G. (2015). Effect of redox conditions on pharmaceutical loss during biological wastewater treatment using sequencing batch reactors. J. Hazard. Mater. 282, 106–115.

Stevens-Garmon, J., Drewes, J.E., Khan, S.J., McDonald, J.A., Dickenson, E.R.V. (2011). Sorption of emerging trace organic compounds onto wastewater sludge solids. Water Res. 45, 3417–3426.

Struijs, J., Stoltenkamp, J., van de Meent, D. (1991). A spreadsheet-based box model to predict the fate of xenobiotics in a municipal wastewater treatment plant. Water Res. 25. 891–900.

Struijs, J. (1996). SimpleTreat 3.0: a model to predict the distribution and elimination of chemicals by sewage treatment plants. RIVM Report 719101025. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

Struijs, J. (2014). SimpleTreat 4.0: a model to predict the fate and emission of chemicals in wastewater treatment plants. RIVM Report 601353005/2014. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

Stuer-Lauridsen, F., Birkved, M., Hansen, L.P., Lützhøft, H.C.H., Halling-Sørensen, B. (2000). Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. Chemosphere 40, 783–793.

Su, L., Aga, D., Chandran, K., Khunjar, W.O. (2015). Factors impacting biotransformation kinetics of trace organic compounds in lab-scale activated sludge systems performing nitrification and denitrification. J. Hazard. Mater. 281, 116–124.

Suarez, S., Lema, J.M., Omil, F. (2010). Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions. Water Res. 44, 3214–3224.

Sui, Q., Huang, J., Deng, S., Chen, W., Yu, G. (2011). Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ. Sci. Technol., 45(8), 3341–3348.

Tan, D., Arnold, W.A., Novak, P.J. (2013). Impact of organic carbon on the biodegradation of estrone in mixed culture systems. Environ. Sci. Technol. 47, 12359–12365.

Tan, D., Arnold, W.A., Novak, P.J. (2015). Estrone degradation: does organic matter (quality) matter? Environ. Sci. Technol. 49, 498–503.

Tchobanoglous, G., Stensel, H.D., Tsuchihashi, R., Burton F.L. (2014) Wastewater engineering: Treatment and resource recovery (5th edition). McGraw-Hill Education, New York, US.

Ternes, T.A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32, 3245–3260.

Ternes, T.A., Kreckel, P., Mueller, J. (1999). Behaviour and occurrence of estrogens in municipal sewage treatment plants – II. Aerobic batch experiments with activated sludge. Sci. Total Environ. 225, 91–99.

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A. (2004a). A rapid method to measure the solid–water distribution coefficient (K_d) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–4084.

Ternes, T.A., Joss, A., Siegrist, H., (2004b). Scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environ. Sci. Technol. 38, 392A–399A.

Ternes, T.A., Giger, W., Joss, W. (2006). Introduction. In Joss, A., Ternes, T.A. (eds.), "Human Pharmaceuticals, Hormones and Fragrances—The challenge of micropollutants in urban water management", IWA Publishing, London, UK.

Ternes, T.A., Bonerz, M., Herrmann, N., Teiser, B., Andersen, H.R. (2007). Irrigation of treated wastewater in Braunschweig, Germany: an option to remove pharmaceuticals and musk fragrances. Chemosphere 66, 894–904.

Terzic, S., Senta, I., Ahel, M., Gros, M., Petrović, M., Barcelo, D., Müller, J., Knepper, T., Marti, I., Ventura, F., Jovančić, P., Jabučar, D. (2008). Occurrence and fate of emerging wastewater contaminants in Western Balkan Region. Sci. Total Environ. 399, 66–77.

Testa, B., Pedretti, A., Vistoli, G. (2012). Foundation review: Reactions and enzymes in the metabolism of drugs and other xenobiotics. Drug. Discov. Today 17, 549–560.

Thiele-Brun, S. (2003). Pharmaceutical antibiotic compounds in soil—a review. J. Plant Nutr. Soil Sci. 166, 145–167.

Tolls, J. (2001). Sorption of veterinary pharmaceuticals in soils: a review. Environ. Sci. Technol. 35, 3397–3406.

Trapp, S., McFarlane, C., Matthies, M. (1994). Model for uptake of xenobiotics into plants: validation with bromacil experiments. Environmental Toxicology and Chemistry 13(3), 413–422.

Trapp, S., Harland, B. (1995). Field test of volatilization models. Environ. Sci. Pollut. Res. 2, 164–169.

Trapp, S., Matthies, M. (1998). Chemodynamics and environmental modelling—An introduction. Springer-Verlag, Berlin Heidelberg, Germany.

Trapp, S. (2004). Plant uptake and transport models for neutral and ionic chemicals. Environ. Sci. Pollut. Res. 11, 33–39.

Trapp, S. (2007). Fruit tree model for uptake of organic compounds from soil and air. SAR QSAR Environ. Res. 18, 367−387.

Trapp, S. (2009). Bioaccumulation of polar and ionizable compounds in plants. In Devillers, J. (Ed.), "Ecotoxicology Modeling", Springer, Berlin, Germany.

Trapp, S., Franco, A., Mackay, D. (2010). Activity-based concept for transport and partitioning of ionizing organics. Environ. Sci. Technol. 44, 6123–6129.

Trapp, S., Eggen, T. (2013). Simulation of the plant uptake of organophosphates and other emerging pollutants for greenhouse experiments and field conditions. Environ. Sci. Pollut. Res. 20, 4018–4029.

Trapp, S. (2015). Calibration of a Plant Uptake Model with Plant- and Site-Specific Data for Uptake of Chlorinated Organic Compounds into Radish. Environ. Sci. Technol. 49, 395−402.

Tülp, H.C., Fenner, K., Schwarzenbach, R.P., Goos, K.U. (2009). pH-dependent sorption of acidic organic chemicals to soil organic matter. Environ. Sci. Technol. 43, 9189–9195.

Ubay Çokgör, E., Sözen, S., Orhon, D., Henze, M. (1998). Respirometric analysis of activated sludge behaviour—I. Assessment of the readily biodegradable substrate. Water Res. 32, 461–475.

Urase, T., Kikuta, T. (2005). Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process. Water Res. 39, 1289–1300.

Vader, J.S., van Ginkel, C.G., Sperling, F.M.G.M., de Jong, J., de Boer, W., de Graaf, J.S., van der Most, M., Stokman, P.G.W. (2000). Degradation of ethinyl estradiol by nitrifying activated sludge. Chemosphere 41, 1239–1243.

van der Ven, A.J.A.M., Mantel, M.A., Vree, T.B., Koopmans, P.P., van der Meer, J.W.M. (1994). Formation and elimination of sulphamethoxazole hydroxylamine after oral administration of sulphamethoxazole. Br J. clin. Pharmac., 38(2), 147–150.

van der Ven, A.J.A.M., Vree, T.B., van Ewijk-Beneken Kolmer, E.W.J., Koopmans, P.P., van der Meer, J.W.M. (1995). Urinary recovery and kinetics of sulphamethoxazole and its metabolites in HIV-seropositive patients and healthy volunteers after a single oral dose of sulphamethoxazole. Br. J. Clin. Pharmac. 39, 621–625.

Vasiliadou, I.A., Molina, R., Martínez, F., Melero, J.A (2013). Biological removal of pharmaceutical and personal care products by a mixed microbial culture: Sorption, desorption and biodegradation. Biochem. Eng. J. 81, 108–119.

Vasiliadou, I.A., Molina, R., Martínez, F., Melero, J.A. (2014). Experimental and modeling study on removal of pharmaceutically active compounds in rotating biological contactors. J. Hazard. Mater. 274, 473–482.

Vasudevan, D., Bruland, G.L., Torrance, B.S., Upchurch, V.G., MacKay, A.A. (2009). pH-dependent ciprofloxacin sorption to soils: Interaction mechanisms and soil factors influencing sorption. Geoderma 151, 68–76.

Vezzaro, L., Benedetti, L., Gevaert, V., De Keyser, W., Verdonck, F., De Baets, B., Nopens, I., Cloutier, F., Vanrolleghem, P.A., Mikkelsen, P.S. (2014). A model library for dynamic transport and fate of micropollutants in integrated urban wastewater and stormwater systems. Environ. Modell. Softw. 53, 98–111.

Vieno, N., Tuhkanen, T., Kronberg, L. (2007). Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res. 41, 1001–1012.

Vieno, N., Sillanpää, M. (2014). Fate of diclofenac in municipal wastewater treatment–A review. Environ. Intern. 69, 28–39.

Vree, T.B., Hekster, Y.A., Baars, A.M., Dansma, J.E., van der Kleijn, E. (1978). Pharmacokinetics of sulphamethoxazole in man: effects of urinary pH and urine flow on metabolism and renal excretion of sulphamethoxazole and its metabolie N4-acetylsulphamethoxazole. Clin. Pharmacokinet. 3, 319–329.

Vree, T.B., Hekster, Y.A. (1987). Clinical pharmacokinetics of sulfonamides and their metabolites – An encyclopedia. Antibiot. Chemother. 37, 1–214.

Vuono, D.C., Benecke, J., Henkel, J, Navidi, W.C., Cath, T.Y., Munakata-Marr, Spear, J.R., Drewes, J.E. (2014). Disturbance and temporal partitioning of the activated sludge metacommunity. ISME J. 9, 425–435.

Walters, E., McClellan, K., Halden, R.U. (2010). Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. Water Res. 44, 6011–6020.

Wang, J.M., Huang, C.P., Allen, H.E. (2000). Surface physical-chemical characteristics of sludge particulates. Water Environ. Res. 72, 545–553.

Weiss, S., Reemtsma, T. (2008). Membrane bioreactor for municipal wastewater treatment – A viable option to reduce the amount of polar pollutants discharged into surface waters? Water Res. 42, 3837–3847.

Wentzel, M.C., Comeau, Y., Ekama, G.A., van Loosdrecht, M.C.M., Brdjanovic, D. (2008). Enhanced Biological Phosphorus Removal. In Henze, M., van Loosdrecht, M.C.M., Ekama, G., Brdjanovic, D., "Biological wastewater treatment—principles, modelling and design", IWA Publishing, London, UK.

Wetzstein, H.G., Schneider, J., Karl, W. (2006). Patterns of metabolites produced from the fluoroquinolone enrofloxacin by basidiomycetes indigenous to agricultural sites. Appl. Microbiol. Biotechnol. 71, 90–100.

Wick, A., Fink, G., Joss, A., Siegrist, H., Ternes, T.A. (2009). Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. Water Res. 43, 1060–1074.

World Health Organization (2014). Antimicrobial resistance: global report on surveillance. Available at www.who.int/drugresistance/documents/surveillancereport/en/

Wu, C., Spongberg, A.L., Witter, J.D. (2009). Sorption and biodegradation of selected antibiotics in biosolids. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 44, 454–461.

Xu, J., Wu, L., Chang, A.C. (2009). Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere 77, 1299–1305.

Xue, W., Wu, C., Xiao, K., Huang, X., Zhou, H., Tsuno, H., Tanaka, H. (2010). Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation. Water Res. 44, 5999–6010.

Yi, T., Harper, Jr, W.F. (2007a). The effect of biomass characteristics on the partitioning and sorption hysteresis of 17α-ethinylestradiol. Water Res. 41, 1543–1553.

Yi, T., Harper, Jr, W.F. (2007b). The link between nitrification and biotransformation of 17α-ethninylestradiol. Environ. Sci. Technol. 41, 4311–4316.

Zhang, D., Zhu, M., Griffith Humphreys, W., (2008). Drug metabolism in drug design and development. John Wiley & Sons, Inc., Hoboken, New Jersey.

Ziels, R.M., Lust, M.J., Gough, H.L., Strand, S.E., Stensel, H.D. (2014). Influence of bioselector processes on 17α-ethinylestradiol biodegradation in activated sludge wastewater treatment systems. Environ. Sci. Technol. 48, 6160–6167.

10 Papers

I **Polesel, F.**, Lehnberg, K., Dott, W., Trapp, S., Thomas, K.V., Plósz, B.G., 2015. Factors influencing sorption of ciprofloxacin onto activated sludge: Experimental assessment and modelling implications. *Chemosphere* **119**, 105–111.

II **Polesel, F.**, Torresi, E., Loreggian, L., Escolá-Casas, M., Christensson, M., Bester, K., Plósz, B.G. (2015). Elimination of pharmaceuticals in pre-denitrifying MBBR – Influence of exposure to primary substrate in single-stage and three-stage configurations. *Manuscript in preparation.*

III **Polesel, F.,** Andersen, H.R., Trapp, S., Plósz, B.G. (2015). Removal of antibiotics in biological wastewater treatment systems – A critical assessment of factors using the Activated Sludge Modelling Framework for Xenobiotics (ASM-X). *Submitted manuscript.*

IV **Polesel, F.**, Plósz, B.G., Trapp, S. (2015). From consumption to harvest: Environmental fate prediction of excreted ionizable trace organic chemicals. *Water Research* **84**, 85–98.

In this online version of the thesis, **paper I-IV** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from.

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